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Kinetics of herbicidal action

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NOTE TO USERS

KINETICS OF HERBICIDAL ACTION

by

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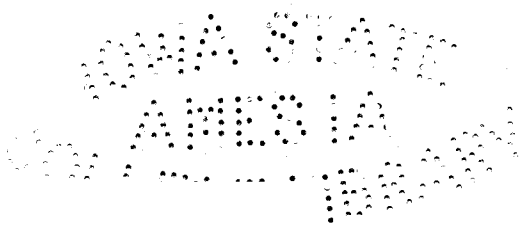

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INTRODUCTION

The basic mechanisms of plant growth, and particularly the mechanism of action of applied and endogenous auxin, have been the object of intensive physiological research for some time. The information gained from many of these studies has led to much conjecture and to the development of many interesting theories regarding growth processes. Increased knowledge concerning such processes has led to the discovery of chemical compounds capable of inducing growth responses in plants similar to those known to be caused by the native growth hormone. Many of these compounds have found application in the agricultural field for such purposes as delaying fruit drop, inducing floral induction, rooting vegetative cuttings of nursery stock, and controlling weeds.

Of the herbicides now in commercial use, many have been shown to function as auxins, which has led to the belief that the growth inhibition induced by these compounds may be intimately connected with the same mechanism or type of mechanisms as auxin-induced growth. Still other herbicides, systemic in nature but not necessarily possessing auxin properties, have also been shown to function by exerting a disruptive action upon the growth mechanism. There is little in the way of concrete evidence, however, that satisfactorily explains the relationship between growth stimulation and

growth inhibition. Likewise, of the many theories developed concerning auxin-induced growth, few are such that quantitative evidence can be offered in their support.

It is the purpose of this investigation to attempt to show that growth inhibition, caused by applied herbicides or growth substances, can be characterized quantitatively by a kinetic analysis of the experimental results. The kinetic analysis is also extended to responses obtained from combinations of growth substances in an attempt to understand more clearly the nature of their interactions in producing growth inhibition.

GLOSSARY

The abbreviations or synonyms of chemical names of compounds used in this investigation are listed as follows:

AT	3-Amino-1,2,4-triazole
Coumarin	1,2-Benzopyrone
2,4-D	2,4-Dichlorophenoxyacetic acid, sodium salt
DCPA	2,2-Dichloropropionic acid, sodium salt
IAA	Indole-3-acetic acid, sodium salt
MH	Maleic hydrazide, sodium salt
TCA	Trichloroacetic acid, sodium salt
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid, sodium salt
TIBA	2,3,5-Triiodobenzoic acid, sodium salt

REVIEW OF LITERATURE

Comprehensive reviews of literature on herbicides have been made by Norman et al. (65), Blackman et al. (15), and Crafts (19). In a recent monograph (50), Leopold surveyed critically the body of knowledge concerning auxins and their functions in plant growth. The present review has been limited to the literature on the mechanism of action of compounds producing growth inhibition or stimulation, and to growth responses resulting from interactions of compounds.

Mechanism of Action

Growth inhibition

Although a tremendous volume of literature has developed in the last decade on the use of herbicides, relatively little work has been done on the fundamental action of these compounds in inhibiting growth. Freed (27) has attempted to summarize knowledge of herbicides other than aryl oxyalkyl acids. Thus it has been found that the enzyme catalase may be inhibited by the presence of chlorate and that arsenic may inhibit respiration by combining with the sulfhydryl groups of dehydrogenases. Phenols are capable of denaturing certain proteins, producing a high rate of respiration in treated plants, while the hydrocarbons in herbicidal oils may reduce the rate of both respiration and transpiration.

Plants treated with substituted ureas often contain increased amounts of ammonia and nitrate nitrogen, while aryl carbamates act as narcotic agents upon mitotic processes. Certain aryl oxyalkanol derivatives such as 2,4-dichlorophenoxyethyl sulfate may give rise to 2,4-dichlorophenoxyacetic acid (2,4-D) in the soil. Crafts (18) has proposed that nonpolar compounds should be used in foliar applications and polar compounds in applications to roots, based on his work showing that nonpolar compounds pass through the cuticle of leaves readily whereas polar compounds enter with difficulty. Currier (21) has studied the responses of plant cells to certain herbicides and has concluded, from observations of vacuolar contraction, vacuolation, granulation and plasmolytic behavior, that irreversible injury is correlated with a marked decrease of cytoplasmic hydration. Blackman (13), in studies of the relative toxicities of herbicides, has concluded that the relationship between percentage mortality and the quantity of toxicant is sigmoidal, and that maximum response rates are obtained at concentrations causing death of half the plant population.

The physiological responses of plants to applications of indole-3-acetic acid (IAA) and naphthaleneacetic acid (NAA) were noted in an early study by Grace (35), who found that high concentrations resulted in injury and death. Foster *et al.* (26), in similar studies, concluded that an auxin

molecule, to be effective in promoting growth, must attach itself by two points to some receptive entity in the plant. They postulated that at high auxin concentrations two molecules might become attached at the two points, each preventing the effective functioning of the other, and that this blocking action might be the cause of herbicidal activity of certain chemicals.

Because of its auxin-like properties at low concentrations, relatively more fundamental work has been done on 2,4-D than on most of the commercial herbicides. Many workers have attempted to correlate the activity of this compound with that of growth hormones produced within the plant. King (47), finding that 50 per cent of 2,4-D-treated water hyacinths could survive because of the formation of new plant parts from basal buds, suggested that killing the tops of the plant broke apical dominance, causing auxin and other substances required for growth to be diverted to basal buds, thus stimulating their growth.

Van Overbeek (86) thought that 2,4-D could move along normal channels of auxin transport and accumulate at active sites of the meristematic protoplasm, thereby upsetting normal auxin functions by interfering with enzymatic processes. He postulated further that 2,4-D, upon combining with suitable proteins, may stimulate the release of inorganic phosphate from phosphorylated compounds, thereby

releasing phosphate-bond energy. This thinking was extended by Van Overbeek et al. (87) in a later paper, where it was concluded that 2,4-D possessed a higher auxin activity than "nonherbicidal auxins", and that it differed from other auxins on the basis of undissociated molecules. The theory was advanced that high auxin concentrations might lead to abnormal accumulation of metabolites such as coumarin derivatives, one of which was more toxic to broad-leaved plants than to grasses. Weintraub (92) proposed a multipoint attachment of auxin to one or more cellular entities in promoting growth, and thought that structurally related compounds such as 2,4-D compete with auxin for these substrates, thereby preventing the auxin action necessary for normal growth. In an attempt to explain the selective effect of 2,4-D, Kvamme et al. (49) studied its action on castor bean lipase and wheat germ lipase. It was found that the inhibitory action on castor bean lipase was 400 times as great as on wheat germ lipase. Freiberg (28) studied the effect of 2,4-D on proteinase and polypeptidase, and found activity decreased in the leaves of treated soybean plants and increased in stems and roots. In further studies on the nature of selectivity, Brian and Rideal (16) found that 2-methyl-4-chlorophenoxyacetic acid (MCPA) would interact with monolayers of certain surface-active compounds such as long-chain amines and ketones, and could be adsorbed on monolayers

produced directly from plant tissue. They suggested that the foundation of species susceptibility could be dependent upon the extent of adsorption of MCPA to sites not concerned in the physiological response, thereby inactivating the compound.

Increased work on auxin relationships in the past few years has brought to the forefront several chemicals of interest, primarily because of their interactions with various growth substances. Thus Audus and Quastel (9) have shown that coumarin inhibited both germination and subsequent root growth and was broadly similar to 2,4-D in differential and formative effects. They concluded that both of these substances act by forming loose combinations or easily dissociated compounds with enzymes or metabolites in the plant cell. In studies on the root growth of pea and cress Audus (7) found that the primary inhibition of growth from low concentrations of both 2,4-D and coumarin was completely reversible, and that the initial rate of inhibition was, possibly, determined by the concentration of growth substance adsorbed at some colloidal interface. Burström (17) studied the effects of coumarin and other compounds on cell walls of wheat roots. He felt that the growth inhibiting properties of coumarin were due to its destruction of the tensibility of the cell wall, making it wholly rigid.

Maleic hydrazide has been found to induce a number of

plant responses which have led to its use both as an herbicide and as an anti-auxin in growth studies. Schoene and Hoffman (73) were the first to report that this compound would inhibit plant growth without causing any obvious morphological abnormalities. Naylor and Davis (63) studied its effect on several plant species, and found that seedlings were most sensitive although growth could be inhibited at any stage. They concluded that maleic hydrazide was transported to regions of meristematic activity where its usual effect was to cause a loss of dominance. Leopold and Klein (51) reported that the compound inhibited the growth of pea roots at concentrations as low as 0.1 ppm, and that 50 per cent inhibition was obtained at 1.0 ppm. They concluded that it was a growth inhibitor, since it was incapable of promoting growth in the absence of auxins. Åberg (3) found that flax roots were not very sensitive to maleic hydrazide, since no inhibition occurred at concentrations less than 10^{-4} M.

The effect of 2,3,5-triiodobenzoic acid (TIBA) on plant growth has been studied by several investigators. Galston (32) described such morphological responses in soybeans as shortening of internodes, loss of apical dominance, epinasty of young leaves and premature abscission of apical leaves and buds. He suggested that TIBA interferes with certain auxin functions within the plant. Wood et al. (97) used a

radioactive form of 2-iodo-3-nitrobenzoic acid, which caused plant responses similar to TIBA, to study translocation in bean and barley plants. They suggested that the compound might enter the plant in molecular form and be translocated as such. Thimann and Bonner (85) concluded that high concentrations of TIBA inhibited growth by excluding auxin from all available growth sites. Waard and Florschütz (91) could find no growth promoting activity of low concentrations of TIBA but did find that it would combine irreversibly with reactive groups in cells of Avena. Although Åberg (3) agreed that TIBA would interact with auxin at low concentrations, he proposed that growth inhibition due to high concentrations was not specifically related to the auxin mechanism.

Growth stimulation

The discovery of chemicals possessing the ability to induce physiological growth responses within plants has led to a wealth of information, including not a little speculation, on the mechanism of auxin action. Although the native growth hormone has not been positively identified, there is considerable evidence that it is indole-3-acetic acid. In any event, it may be assumed that gross responses resulting from exogenous synthetic auxins can be closely enough correlated to known plant responses due to endogenous growth hormones to allow more or less accurate measurement of these

effects. No attempt will be made here to cover the entire historical development of knowledge concerning auxin mechanism; rather, some of the more recent findings and theories will be reviewed.

Grace (35) was able to establish that treatment of seeds and young lettuce and tomato plants with IAA and NAA and their derivatives would result in stimulation of growth. Sweeney and Thimann (82) found that the addition of malate to sub-optimal concentrations of IAA increased protoplasmic streaming in Avena, and concluded that this strengthened the relationship between respiration and growth since malate is also necessary in respiration. Determinations of decreases of protoplasmic viscosity in cortical cells of stems and petioles of beans treated with IAA, NAA, and indole-3-propionic acid, led Northen (66) to believe that such decreases were caused by dissociations of cellular proteins and would result in alteration of plant development. Further work by Wildman and Gordon (96) has shown that auxin is associated with proteins isolated from spinach leaves and that it can be released by enzymatic hydrolysis.

Enzyme activation has been suggested by Berger and Avery (12) as being the most likely mechanism of auxin action. They found that alcohol dehydrogenase was closely associated with growth and that its activity was increased in the presence of IAA. Eyster (24) found that IAA and NAA

retarded the action of isolated diastase and accelerated the action of diastase adsorbed on charcoal. It was suggested that release of enzymes from adsorption surfaces would result in growth promotion and that the effect on enzyme alone would result in growth inhibition. Tang and Bonner (83) found that IAA was constantly being inactivated in etiolated pea epicotyls by an enzyme which showed great specificity for IAA.

Surface activation of cell membranes has been considered by Veldstra (88) to constitute the major function of auxins. He visualizes the growth substance as an "opening factor", increasing permeability of cell membranes to such things as water and possibly sugars. Veldstra and Booij (90) postulate further that increasing the lipophilic character of a compound may cause it to interact too strongly with the membrane, thus preventing optimal activity. Paleg and Muir (68), however, concluded that chemical reactivity was more important than adsorbability as a measure of growth-inducing properties. They measured the suppressive effect of growth substances upon the polarographic oxygen maximum at a phase boundary, and could find no correlation of surface activity with physiological activity. Linser and Kaindl (53) visualized plants as containing both growth-promoting and growth inhibiting substances which were able to occupy two kinds of active "spaces", one type giving rise to growth stimulation and the other to growth inhibition.

The metabolization of phenoxyacetic acids or other growth promoting substances to generate energy-rich phosphate bonds in the plant was suggested by Rhodes and Ashworth (71) as a possible mechanism of activity. They indicated that the energy contained in these bonds might be the major factor in the initiation of the growth response. French and Beevers (29) extended this concept by postulating that the respiratory stimulation induced by both IAA and 2,4-D resulted from an increased internal supply of energy-rich phosphate bond acceptors and was, therefore, a consequence of the stimulation of growth. Audus (8) examined some of these theories on auxins and verified the hypothesis that respiration was stimulated by auxin in Avena coleoptiles. He criticized much of the recent thinking, however, as being too restricted and superficial. In the same work the theory was advanced that auxin may act to stimulate the first phase of wall stretching in cell elongation by increasing the swelling capacity of the inter-micellar colloids. Burström (17), however, pictured this first phase of cell elongation as a shift from an irreversible plastic to an elastic tensibility by means of a reversible dissolution of the cell wall, mediated by auxin.

There have been several attempts to relate chemical structure to physiological activity. Thus Koepfli et al. (48) described the following minimum structural requirements

for stimulation of cell elongation in higher plants: (a) a ring system as nucleus, (b) a double bond in this ring, (c) a side chain, (d) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least one carbon atom removed from the ring, and (e) a particular space relationship between the ring and the carboxyl group. These requirements were later modified somewhat by Went (94) by stating that the side chain had to be adjacent to the ring double bond and at least two carbon atoms in length. The work of Skoog et al. (76) verified the requirement for a certain structural configuration, but added the condition that there must be a reactive group of specific chemical nature. They suggested that these requirements could be used to determine the relative activity of different auxins. Åberg (4), in characterizing auxin antagonists, agreed with the essentiality of a ring structure combined with a side chain, and suggested that there were properties of the side chain of the antagonist or steric relations to the ring that made them unfitted for auxin action. Muir and Hansch in a series of papers (62, 38, 61, 60) brought out the concept in which it was assumed that the ortho position on the ring adjacent to the side chain was directly involved in the growth reaction (62) and that growth substances of the phenoxyacetic and phenylbutyric acid types reacted with a plant substrate through an ortho position (38). It was further postulated

that cell elongation of Avena coleoptile sections could be promoted only by substituted benzoic acids with an electronegative atom or group capable of displacement by an electron-rich substrate in one or both ortho positions (61). In tests involving 117 compounds, using the cell elongation technique, they concluded that the ortho reaction hypothesis was valid in most cases, although there were a few compounds that did not fit their concept (60). The work of Thimann (84) did not wholly agree with the ortho concept, since it was found that certain derivatives of phenoxyacetic acid were active as auxins in the pea test when substituted in both positions ortho to the side chain. He concluded that a definite chemical reaction could take place at one or more of the positions on the ring.

The concept of a two-point attachment of an auxin molecule with some receptor entity within the cell is fundamental to the theory of auxin action presented in a series of papers by McRae, Foster and Bonner (26, 56, 57, 58). They have postulated that the molecule combines with the receptor by means of the carboxyl group and a position on the ring ortho to the side chain, and that the side chain must be of a spatial relationship such that the two-point attachment would not be prevented by steric hindrance of the side chain. These workers have attempted to make these relationships understandable in the light of classical enzyme kinetics as

first postulated by Michaelis and Menten (59) and later elaborated by Lineweaver and Burk (52). Thus, according to these concepts, auxin would combine reversibly with the receptor or substrate to form an auxin-substrate complex which would then decompose irreversibly into final products, ultimately giving rise to growth. These workers have applied these concepts quantitatively to the extension-growth of Avena coleoptile sections exposed to auxin solutions for twelve hours, a period of time for which they consider growth of the sections to be linear. This measurement of growth is then assumed to be an estimate of the initial growth velocity, and, when plotted against auxin concentration, describes a hyperbola. The mathematical expression of the hyperbola yields two constants: the maximum velocity (V_{max}) of the growth reaction, or the velocity attained when all the substrate is saturated with auxin, and the dissociation constant of the reversible reaction (K_s), which is the auxin concentration at which one-half maximum velocity is attained. If this system truly follows the Michaelis-Menten kinetics, a straight line will result when the reciprocal of the initial velocity is plotted against the reciprocal of the auxin concentration (52). Data presented in these papers indicate that these may indeed be the true relationships existing within plants. Complete approval has not been accorded this analysis of auxin action, however. Audus (6), for one, has

questioned the advisability of attempting to relate complex growth systems to simple enzymic processes. Bennet-Clark and Kefford (11) objected to the assumption that extension growth for a 12 hour period is a true estimate of the initial rate of reaction, and maintained that the growth of coleoptile sections supplied with high concentrations of IAA decreases rapidly with time. Housley et al. (45), on the other hand, concluded that the kinetic analysis could be validly applied to the system controlling cell elongation in coleoptile sections, but that intracellular substrate concentrations were regulated by active transport and permeability of cells to applied auxin. Their data did not support the theory that inhibition resulted from steric hindrance of two-point attachment but indicated that it might be due to a toxic action of auxin on cell protoplasm. Leopold (50), in reviewing the principles of the kinetic analysis, feels that it is the best explanation advanced thus far, but cautions that the velocity reaction may be controlled by steady state conditions rather than an equilibrium reaction.

Interactions

There have been numerous attempts to evaluate growth responses to applications of mixtures of various compounds. In the herbicidal field the desired goal might be increased efficiency in the eradication of a particularly troublesome

weed species, or an attempt to bring under control a varied weed population with a single application of chemicals. In physiological studies of growth, mixtures of compounds have been employed to elucidate the mechanism of auxin action. There are three generally accepted types of response to be obtained from combinations: (1) additive, wherein the level of response obtained from combinations of chemicals is the sum of their separate action, (2) antagonistic, where the resultant response is less than might be expected from the action of the constituents alone, and (3) synergistic, where the combined activity of the two or more components exceeds that expected from the sum of the components used alone (25, 72, 80).

A synergistic effect has been claimed by Hance (36, 37) in the use of sodium pentachlorophenate as an "activator" of herbicides. Dancaster (22) reported that the effect of sodium chlorate could be enhanced by the addition of such catalysts as manganese, cobalt and nickel salts, and vanadium pentoxide, which by themselves had no killing effect. Sen and Woodford (74) found that the normal inhibitory effects of trichloroacetic acid (TCA) on growth of pea shoot segments could be reversed by 2,4-D, and that growth might even be accelerated. They suggest that herbicidal mixtures of TCA and 2,4-D might prove disadvantageous. Crafts (20) and Mangual (55) have suggested the addition of 2,4-D to oil and

sodium pentachlorophenate emulsions as an aid in killing commelina and nutgrass in the tropics. Nolla (64) has found that 2,4-D dissolved in diesel or aromatic oil is a much more effective grass herbicide than either alone.

Allard et al. (5) found that a mixture of either 2,4-D or 2,4,5-T with isopropyl-N-phenylcarbamate was an effective herbicide against seedlings of both broadleaf and grass species but that there were no clear-cut interaction effects. King (47) found that the addition of phenylacetic acid to solutions of 2,4-D was an effective way of preventing regrowth of basal buds of water hyacinths which were only incompletely controlled by 2,4-D alone. Lucas and Hamner (54) reported a significant increase in the herbicidal action of 2,4-D from the addition of onion extract. Spear and Thimann (77) found that onion juice would increase the growth promoting activity of IAA and 2,4-D in both the curvature of split pea stems and straight growth of pea stem sections, and concluded that the action of onion juice was due to its content of sugar, phosphate and potassium ions. It was reported by Hitchcock and Zimmerman (42) that mixtures of 2,4-D with such compounds as ammonium thiocyanate, ammonium sulfamate, arsenicals, diallyl maleate, sodium bicarbonate and sodium chloride, resulted in more effective herbicides than any of the individual components used at the same concentration as in the mixture. Stewart et al. (79) found that leaf and fruit drop

could be inhibited and fruit yields of citrus increased by adding 4-8 ppm of 2,4-D to insecticidal oils, with no apparent phytotoxic effects.

Much of the current literature on the influence of auxins in promoting growth has been stimulated by the discovery of chemicals capable of altering the response to applied auxin. Our present knowledge of auxin action has largely been gained through the employment of certain of these chemicals in combinations with auxins. Thus Skoog et al. (76) found that phenylbutyric acid inhibited the activity of IAA and postulated that the action might be competitive. These workers believed that auxins might act as co-enzymes. Veldstra and Booij (90) objected to this view, and believed rather that they functioned as regulators of enzymatic activity. Veldstra (89) considered that analogues of compounds active in growth may compete with the active compound for "waste" sites on which the primary compound would otherwise be inactivated or used up. The primary active compounds would then be relatively more available for their essential function, a type of competition that he considers synergistic. Along this same line of reasoning Thimann and Bonner (85) have shown that TIBA enables a small amount of auxin to bring about a disproportionate amount of growth. This was interpreted to mean that TIBA is sufficiently like auxin in structure to be able to combine with the

same substrate and, in low concentrations, leave open a small but optimum number or distribution of sites with which auxin can combine and promote growth with a small number of molecules. High concentrations of TIBA gave growth inhibition, presumably due to the exclusion of auxin from growth sites; a result which is in essential agreement with the findings of Galston (32) and Waard and Florschütz (91). The latter investigators, however, could find no evidence of a growth promoting activity of TIBA in low concentrations. Åberg (3), on the other hand, claimed that low concentrations of TIBA had a synergistic effect on both native auxin and applied IAA in the root growth of flax seedlings. Audus (6) suggested that this effect might be additive rather than synergistic.

Åberg (3) further showed that maleic hydrazide may exhibit weak antiauxin effects with IAA, which he thought involved a mechanism of accelerated auxin destruction. Leopold and Klein (51), in tests with pea roots, have also shown that maleic hydrazide acts as an anti-auxin, since it acts in opposition to auxin in growth.

Growth interactions between IAA and nicotinic acid have been studied by Galston (30, 31) using etiolated pea epicotyls. He concluded that nicotinic acid was involved in the growth effects produced in the plant by IAA, and suggested that both originate from a common precursor, tryptophan.

Went (93) found that mixtures of low concentrations of IAA and 2,4-D gave a response in the pea test more than twice that to be expected from additive effects, and concluded that the compounds were participating in two separate, supplementary reactions. Goldacre (33) postulated that IAA was constantly being inactivated in plants by an enzyme, IAA oxidase, and that this rate of destruction was increased by the addition of 2,4-D. It was later shown by Goldacre *et al.* (34) that the increased rate of destruction was due to 2,4-dichlorophenol, frequently present in 2,4-D as an impurity, and that pure 2,4-D was without effect on the enzyme. Hitchcock and Zimmerman (41, 43) have concluded from their studies of IAA and 2,4-D on tomatoes that activation or inhibition of 2,4-D did not depend upon the structural specificity of the activator or inhibitor. They found that IAA functioned as an inhibitor of 2,4-D. Blackman and Robertson-Cuninghame (14) studied the interaction of IAA and 2,4-D on growth of *Lemna minor* and *Helianthus annuus*. They found that the two compounds could interfere with one another and proposed that the action was competitive. They believed that there was more than one mechanism involved, however.

Other interactions reported with growth substances include the work of Hartman and Price (39) who found more severe effects when bean plants infected with Southern Bean

Mosaic were treated with beta-naphthoxyacetic acid than were obtained with the growth substance or virus alone. Steward and Caplin (78) reported that mature potato tuber parenchyma cells could be successfully cultured only when a combination of coconut milk and 2,4-D was added to the mixture, an effect they termed synergistic. Shantz et al. (75) later found that other substances, such as alpha-(2-naphthoxy)- and alpha-(2,4,5-trichlorophenoxy)-propionic acids, could replace the 2,4-D in the combination. Osborne (67) believed that 3-indoleacetonitrile isolated from Brussels sprouts was changed to IAA in the presence of Avena coleoptiles. The addition of nitrile to the acid increased curvature of split peas approximately 30 times over higher concentrations of the acid alone, an effect also thought to be synergistic. Parry (69) found a synergistic action when mixtures of thiourea and IAA were applied to germinating Avena seedlings. Åberg (1, 2, 4) studied the effect of a variety of mixtures on the root growth of flax seedlings. Thus a low concentration of 1-naphthol restored growth when added to an inhibiting concentration of naphthaleneacetic acid and was, therefore, thought to be an auxin antagonist. Certain other naphthalene derivatives were found to stimulate growth of roots when applied alone, and to restore root growth inhibited by 2,4-D. Phenylacetic, gamma-phenylbutyric, cyclohexylacetic, and (+)-alpha-phenoxypropionic acids were found

to function as weak auxins, while phenoxyacetic and alpha-phenoxyisobutyric acids were found to function as auxin antagonists, with an ability to counteract the inhibition caused by 2,4-D.

McRae, Foster and Bonner (56, 57, 58) have extended their kinetic studies to include investigations of the mechanism of anti-auxins. They have shown that an auxin inhibitor will combine with the receptor entity to form an inhibitor-receptor complex but that this complex undergoes no further reaction. When the reciprocal of growth is plotted against the reciprocal of concentration, the presence of an inhibitor causes the slope of the line to be higher, although the intercept remains unchanged. Inhibitors giving this type of response are competitive with auxins, but their effects in low concentrations are alleviated by increasing auxin concentrations. A relationship was postulated between auxins on the basis of their calculated constants. Thus auxins characterized by low $\underline{K_s}$ and low $\underline{V_{max}}$ are capable of inhibiting or augmenting activity of auxins of similar $\underline{K_s}$ and higher $\underline{V_{max}}$. Results obtained with these methods supported the findings of Hoffman (44) that 2,4,6-trichlorophenoxyacetic acid competitively and reversibly inhibits the action of 2,4-D and IAA. It was found that 2,6-dichlorophenoxyacetic acid and 2,4-dichloroanisole functioned in like manner (57). It is considered that such anti-auxins

are derived from an active auxin by (1) elimination of the essential carboxyl group, (2) elimination of the essential reactive ortho group or (3) elimination of proper spatial relationships, as with 2,4-dichlorophenoxyisobutyric and 4-chlorophenoxyisobutyric acids which introduce bulky groups in the side chain. They postulated that a compound considered active as an anti-auxin may be capable of combining with the substrate at one point but not two (56). Chemically different auxins such as IAA, NAA and 2,4-D are considered to compete with one another for auxin-receptive sites within the plant (58).

Audus and Shipton (10) disagreed with the conclusion that 2,4-dichloroanisole was a true anti-auxin, since they found it only very weakly antagonistic to 2,4-D inhibition of cress root growth. Audus (6) also criticized the idea of an anti-auxin acting at the growth site. He considered that if native auxin had been controlling growth, anti-auxins should have reduced the growth of the controls. Ingestad (46), using a kinetic analysis, found that phenoxyacetic acid did counteract both native and exogenous auxin. He felt that competition occurred in the step from activation of native auxin to growth. Although work of McRae and Bonner (57) indicated that the value for the inhibition constant (K_I) of a given inhibitor was independent of the auxin present, Housley *et al.* (45) showed that this was not

necessarily true. They also attacked the theory that inhibition resulted from steric hindrance of two-point attachment.

An examination of the literature reviewed here can only lead to the conclusion that our knowledge of the mechanism of action of applied growth substances is far from complete. Of the many theories advanced, agreement among investigators as a whole can be reached only on points of secondary interest. This is a clear indication of the need for more exacting research in this important field.

MATERIALS AND METHODS

Experiments with Soybeans

Soybeans of the Hawkeye variety constituted the experimental plant material used in the greenhouse studies. The seedlings were grown in 4-inch pots with three plants in each. Individual pots were considered to be the experimental unit. The plants were treated at the time the primary leaves were fully expanded but while the first trifoliates were still in the bud. The plants in single pots were sprayed on a turntable rotating at 78 rpm to insure uniformity of application (40). All herbicides, growth substances, and mixtures of compounds were applied in aqueous solutions with 0.1 per cent of Tergitol 7 wetting agent added to each solution. Ten milliliters of solution were applied to the plants in each pot by means of a modified De Vilbiss paint spray gun operating at a constant pressure of 12 psi. The pots were arranged in randomized block designs and 6 to 8 replicates were used throughout.

The green-weight method of evaluating toxicity was used for these studies (23, 42). Growth after treatment was considered to be a function of the concentration of chemical applied and was measured as the fresh weight of all plant material above the primary leaves at 10 to 14 days after

treatment. Due to inherent and uncontrollable variation between experiments, the average fresh weight of plant material for each concentration was converted to percentage reduction of growth from controls.

Experiments with Yeast

Cultures of Saccharomyces cerevisiae were grown in nutrient media and the growth determined turbidimetrically according to methods first described by West and Henderson (95) and later modified by Pool (70) and Swanson (81).

Agar nutrient slants were inoculated with yeast cells from the center of a pound cake of Fleischmann's bakers' yeast and incubated for 24 hours at 30° C. The slants were then stored at 5° C. Stock cultures were inoculated from the slants and incubated in 125 ml Erlenmeyer flasks for 24 hours at 30° C. Each stock culture was subcultured three times before use in the experimental cultures. The solution used for subculturing consisted of 25 ml of distilled water and 25 ml of a double strength nutrient solution made up as follows:

20 g glucose
3 g ammonium sulfate, $(NH_4)_2SO_4$
2 g potassium acid phosphate, KH_2PO_4
0.25 g calcium chloride, $CaCl_2 \cdot 2H_2O$

0.25 g magnesium sulfate, $MgSO_4 \cdot 7H_2O$

2.5 g yeast extract (Difco)

distilled water to make 500 ml.

All flasks containing the nutrient media used for stock cultures and experimental cultures were plugged with cotton and autoclaved for 20 minutes at 15 pounds pressure before inoculation. A sterile inoculating loop was used for transferring the yeast cells from one flask to another.

Experimental cultures were incubated in 50 ml Erlenmeyer flasks for 15 hours at 30° C. after inoculation. The media in the experimental flasks consisted of 10 ml of double strength nutrient solution of the same composition as described in the preceding paragraph, and 10 ml of a double strength aqueous solution of herbicide or growth substance. The controls consisted of 10 ml of distilled water plus 10 ml of the double strength nutrient solution. A blank was included with each experiment and was identical with the controls except that it was not inoculated. Duplicate cultures were used for all treatments in these experiments. This was considered a sufficient number since good agreement was found between the two samples for each treatment. The coefficients of variability for these experiments were low, ranging from about 2 to 4 per cent.

The flasks were shaken periodically during the 15 hours. At the end of this period each flask was again shaken

thoroughly to insure complete mixing and suspension of the yeast cells in the medium. Approximately 5 ml of each culture was poured into standard colorimeter test tubes and a reading was taken immediately using a Klett-Summerson photoelectric colorimeter with a red (M660) filter. The colorimeter tubes used in these studies were selected by checking them against a standard and discarding those which varied more than one per cent.

West and Henderson (95) made the assumption that the growth of the yeast was directly proportional to the concentration of cells as measured by the turbidity of the cultures. This assumption was found to be correct in the present investigation by correlating optical density with cell counts obtained with a haemacytometer over a 24 hour period. The two measurements were found to be directly proportional to one another for a period of time well in excess of 15 hours. The colorimeter scale readings (optical density x500) were used in these studies as a relative measure of growth.

The 15 hour period was chosen as the growth period for these studies after plotting growth against time graphically (Fig. 1). An attempt was made in this investigation to study the kinetics of growth inhibition. This necessitates obtaining an estimate of the initial velocity of growth, which is best expressed by that portion of the curve where growth is

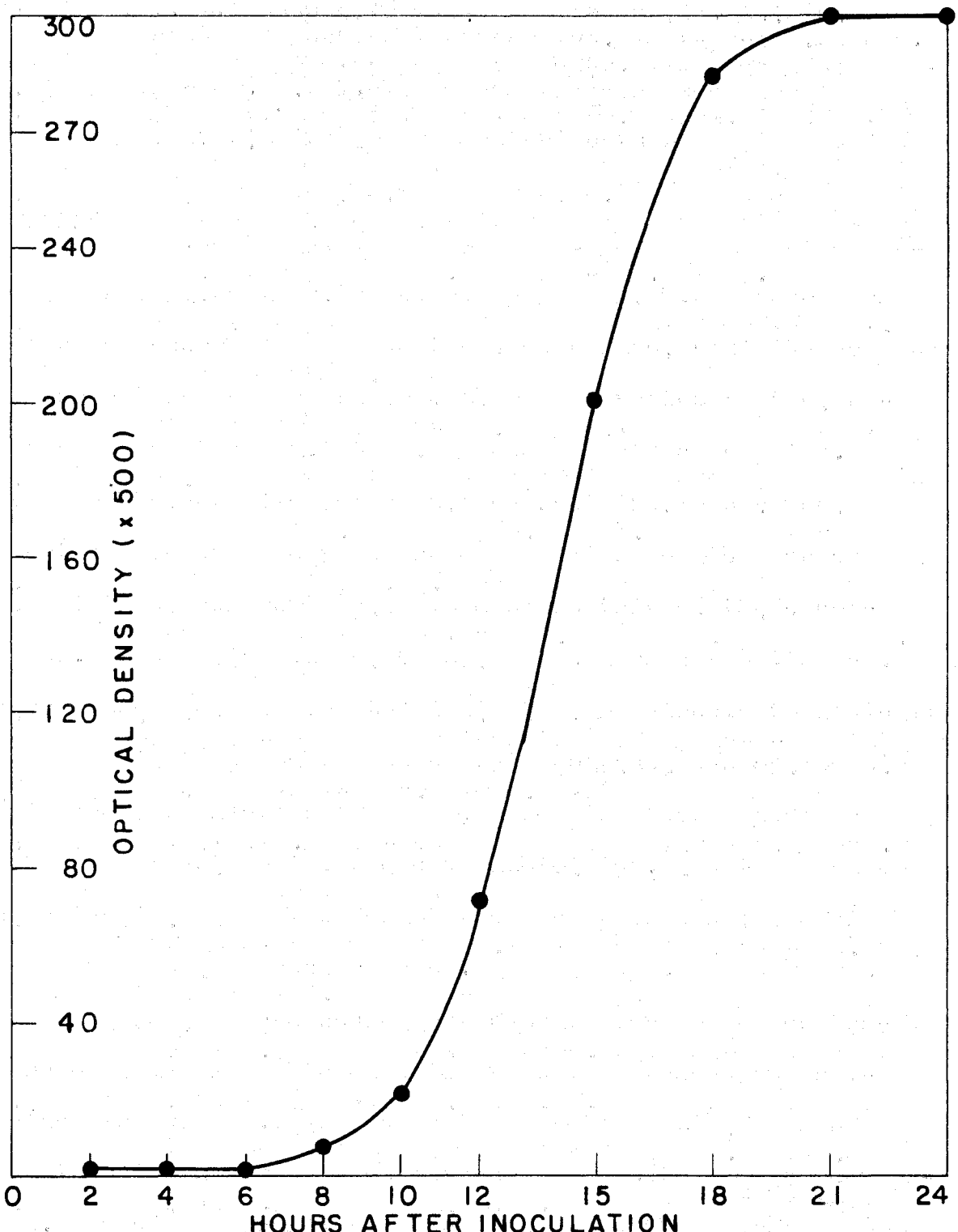

Fig. 1. Rate of growth of yeast measured turbidimetrically in a 24-hour period

linearly related to time and before environmental factors have begun to limit it. It will be noted from the graph that the 15 hour time interval falls within this range. All growth curves studied showed certain similarities in that the rate of growth was very slow for a period of 6 to 8 hours after inoculation and that growth was nearly linear from approximately 8 to 15 hours after inoculation. It seems reasonable, therefore, to consider 6 to 8 hours after inoculation as the time at which inhibition starts and measurements made at 15 hours as good estimates of the initial velocity of growth inhibition.

EXPERIMENTAL RESULTS

Single Chemical Experiments

Experiments with soybeans

Soybean seedlings, when treated in the greenhouse and harvested according to the methods described, exhibit growth responses which are considered to be a function of the concentration of the herbicide or growth substance applied. An accurate characterization of these growth responses was undertaken in an attempt to shed more light on the mechanism of inhibition of growth exhibited by certain of these compounds. The ultimate goal of these studies was to find a means of determining how the inhibition obtained with a single compound might be modified when this compound was applied in a mixture with another compound, which in itself might or might not be active in producing inhibition. To this end a determination of the action of these compounds applied singly was felt to be a necessary step.

The growth responses of soybeans to varying concentrations of several herbicides and growth substances are summarized in Table 1. An examination of the data indicated that inhibition might be expressed as a function of concentration. An attempt was then made to express this function mathematically. The results obtained with 2,4-D will be used

Table 1. Growth of soybean seedlings expressed as increase of fresh weight (g), and percentage inhibition (I) of growth above primary leaves 10 to 14 days after application of growth substances (average of 8 reps.*)

Growth substance	Conc.	Fresh wt.	% I
2,4-D	M x 10^{-4}		
	0.00	3.10	00.0
	0.32	2.43	21.6
	0.64	2.08	32.9
	1.28	1.54	50.3
	2.56	0.97	68.7
	5.12	0.38	87.7
	10.24	0.15	95.2
	20.48	0.06	98.1
DCPA	M x 10^{-3}		
	0.0	2.68	00.0
	0.5	1.73	35.4
	1.0	1.36	49.3
	2.0	0.86	67.9
	4.0	0.46	82.8
	8.0	0.14	94.8
	16.0	0.02	99.3
2,4,5-T	M x 10^{-6}		
	0.0	3.88	00.0
	2.0	2.98	24.3
	4.0	1.91	39.1
	8.0	1.60	56.3
	16.0	1.62	72.0
	32.0	0.53	83.7
	64.0	0.12	91.1
	128.0	0.04	95.4

*Samples of complete data are shown in Tables 14, 15, 16 and 17 in the appendix.

Table 1. (Continued)

Growth substance	Conc.	Fresh wt.	% I
TCA	M x 10^{-3}		
	0.0	1.39	00.0
	0.5	0.91	33.3
	1.0	0.69	50.0
	2.0	0.40	66.7
	4.0	0.13	80.0
	8.0	0.07	88.9
	16.0	0.03	94.1
AT	M x 10^{-3}		
	0.0	2.10	00.0
	0.25	1.74	26.5
	0.5	1.21	41.9
	1.0	0.63	59.0
	2.0	0.37	74.2
	4.0	0.10	85.2
	8.0	0.06	92.0
	16.0	0.01	95.8
IAA	M x 10^{-3}		
	0.0	3.80	00.0
	0.25	1.96	48.4
	0.5	2.24	41.1
	1.0	1.81	52.4
	2.0	1.40	63.2
	4.0	1.24	67.4
	8.0	0.56	85.3
MH	M x 10^{-3}		
	0.00	1.65	00.0
	0.72	1.39	16.0
	1.44	1.21	27.5
	2.88	0.82	43.2
	5.76	0.70	60.3
	11.52	0.49	75.2
	23.04	0.39	85.9

to illustrate the development of these relationships.

It was found that when percentage inhibition was plotted against concentration, a hyperbola of the type shown in Fig. 2 resulted. This hyperbola can be described by the equation

$$c - y = \frac{x}{a + bx} \qquad (1)$$

in which $c - y$ is the percentage inhibition (c = 100 per cent growth and y is the percentage value of growth for each concentration x) and a and b are constants.

The reciprocal of equation 1 may be written as follows:

$$\frac{1}{c - y} = a \cdot \frac{1}{x} + b \qquad (2)$$

Plotting the reciprocal of the percentage inhibition ($\frac{1}{c - y}$) against the reciprocal of the concentration ($\frac{1}{x}$) results in a straight line of the type shown in Fig. 3. The transformation of the data into a linear relationship is especially significant in that it provides a means of deriving constants, and may also be useful in allowing accurate prediction of the probable action of the chemical in mixtures with other compounds. Furthermore, the equations for the hyperbola and the straight line provide a good mathematical basis for the development of hypotheses concerning these results in physiological terms. The hypothesis developed here was adopted by analogy from classical enzyme kinetics which affords a means

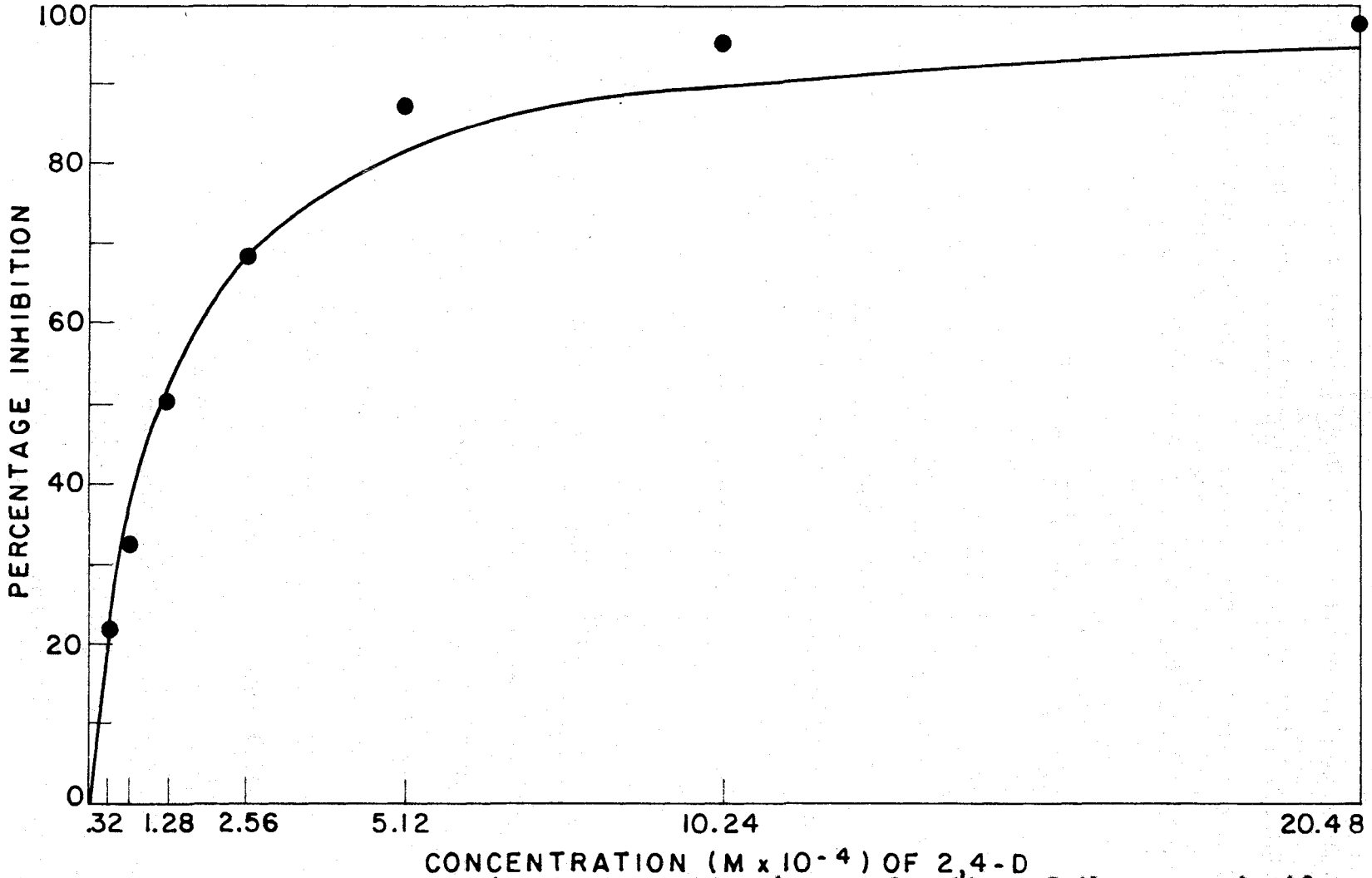

Fig. 2. Inhibition of growth (soybean seedlings) as a function of the concentration of 2,4-D. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of fresh weights

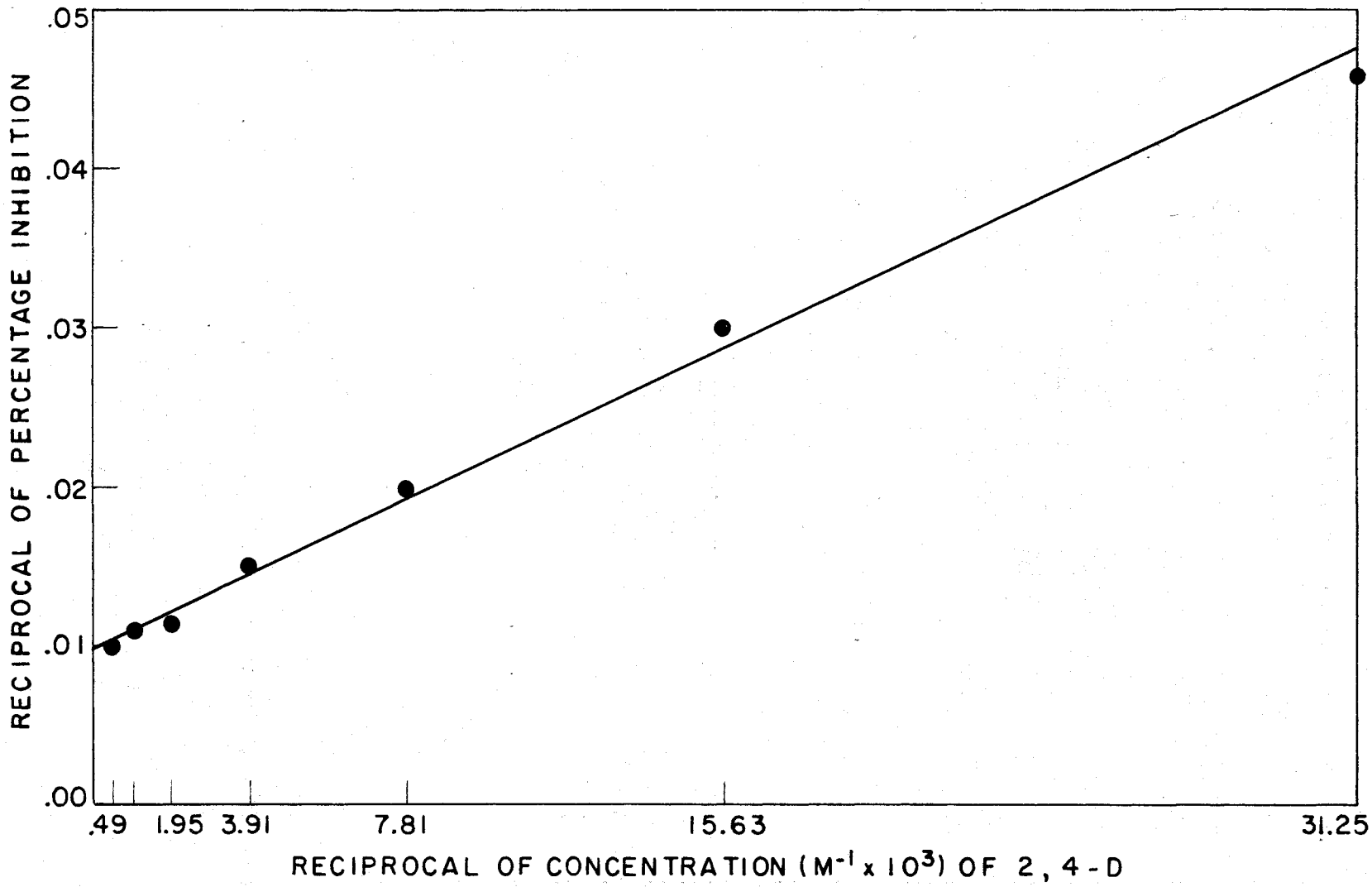

Fig. 3. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of 2,4-D. Calculation of linear regression yielded these values for the constants: $V_{max} = 100$, $K_m = 1.18 \times 10^{-4}$

of determining the type of interaction occurring among an enzyme, an enzyme inhibitor and an enzyme substrate. Although this kinetic treatment was originally formulated for enzyme systems, it is not to be implied that its application here is an indication that the reactive sites with which the herbicide or growth substance combines within the plant are necessarily enzymic in nature. The structure of these sites may very possibly be intimately connected with certain enzyme systems but it must be emphasized that the data allow nothing more than speculation concerning these sites.

It is postulated that a herbicide (H) will combine reversibly with some mechanism or reactive site (M) within the plant to form a herbicide-mechanism complex (HM). This complex is then thought to be transformed irreversibly into products which ultimately give rise to inhibition of growth. The above concepts may be expressed by the following equation:

$$H + M \underset{k_2}{\overset{k_1}{\rightleftharpoons}} HM \xrightarrow{k_3} \text{Inhibition of Growth} \qquad (3)$$

in which k_1, k_2, and k_3 are rate constants of the two reactions. The general dissociation constant of the reversible reaction may be expressed as follows:

$$K_m = \frac{(H)\ (M)}{(HM)} \qquad (4)$$

in which (H), (M), and (HM) are concentrations of the herbicide, mechanism, and complex, respectively. The initial velocity of the irreversible reaction is a function of the concentration of the complex and can be expressed in this manner:

$$v = k_3 (HM) \qquad (5)$$

A quantitative measure of the hypothesis given in equation 3 may be obtained from the Michaelis-Menten equation for the initial velocity of the growth inhibition. This equation is

$$v = \frac{V_{max}(H)}{K_m + (H)} \qquad (6)$$

in which v is the initial velocity, (H) is the molar concentration of the herbicide, V_{max} is a constant representing the maximum velocity of the growth inhibition, attained when all the receptor sites or mechanisms are saturated with the herbicide, and K_m is the equilibrium constant of the reversible reaction, or the concentration of the herbicide when one-half the maximum velocity is attained. In these experiments the measure of growth 10 to 14 days after treatment is considered to be a measure of the initial rate of growth inhibition. It is known from previous experience that soybeans grown and harvested using these techniques exhibit a growth rate that is nearly linear over the period used.

Equation 6 may be rewritten in the reciprocal form (Lineweaver and Burk) as follows:

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{(H)} \qquad (7)$$

A plot of the reciprocal of the initial velocity (which is the same as $\frac{1}{c - y}$ in equation 2) against the reciprocal of the concentration results in the straight line depicted in Fig. 3. This straight-line plot now affords a means of evaluating the constants V_{max} and K_m. The intercept of the line on the ordinate is $1/V_{max}$ and the slope of the line is K_m/V_{max}. The value for the intercept may be obtained directly from the graph and substituted in the expression

$$\frac{K_m}{V_{max}} = \text{slope} \qquad (8)$$

to get the value for K_m. In these experiments, however, the line was fitted to the experimental points by calculation of linear regression, from which the values of the slope and intercept were obtained.

Equation 6 is also an expression of the hyperbola shown in Fig. 2. When the constants, derived from the double reciprocal plot, are inserted in equation 6, new values for the percentage inhibition for each concentration of the herbicide can be calculated. These values are found to correspond closely to the experimental values as shown in Fig. 2 where

the continuous line represents the calculated percentage inhibition and the points represent the experimental values. The data and the calculations used in presenting Figs. 2 and 3 are summarized in Table 2. The graphical representation of the data for 2,4-D indicates that these relationships hold true over the range of concentrations investigated.

This treatment has been extended to include several systemic herbicides and growth substances. Thus, in Figs. 4 and 5, the effect of DCPA on the growth of soybeans is

Table 2. Inhibition of growth of soybeans by 2,4-D. Sample of calculated data used in graphical presentation

Conc. (x) $\underline{M} \times 10^{-4}$	Av. wt. growth (g)	% inhibition* (c - y)	$\frac{1}{c - y}$	$\frac{1}{x}$ ($\times 10^3$)	Calc. % inhibition**
0.00	3.10				
0.32	2.43	21.6	0.046	31.25	21.3
0.64	2.08	32.9	0.030	15.63	35.2
1.28	1.54	50.3	0.020	7.81	52.0
2.56	0.97	68.7	0.015	3.91	68.5
5.12	0.38	87.7	0.011	1.95	81.3
10.24	0.15	95.2	0.011	0.98	89.8
20.48	0.06	98.1	0.010	0.49	94.5

*c = 100% growth, y = growth as percentage of control.

**From equation 6. $\underline{V_{max}} = 100$, $\underline{K_m} = 1.18 \times 10^{-4}$.

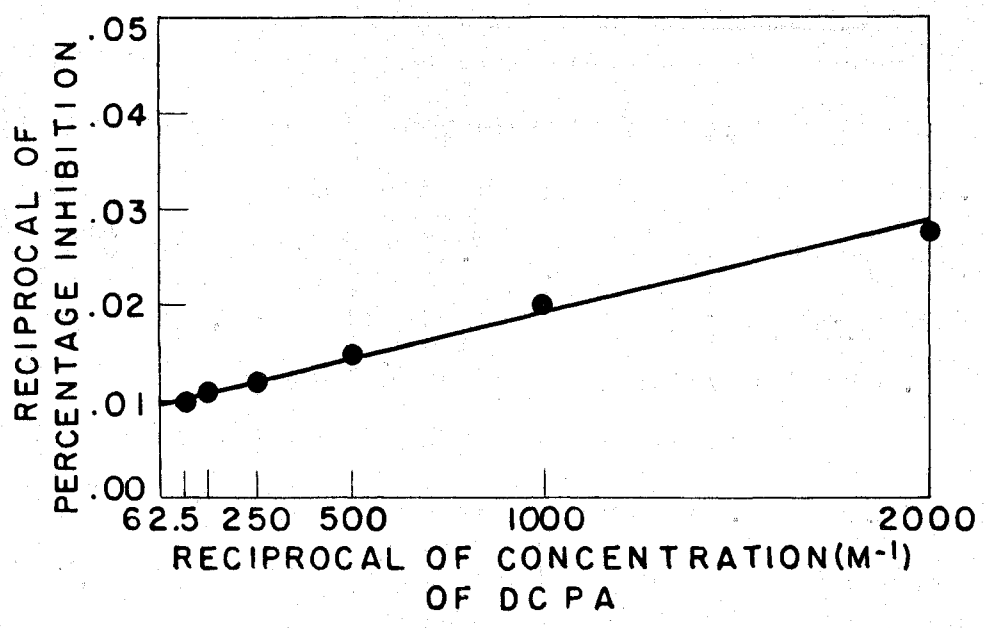

Fig. 4. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of DCPA. Calculation of linear regression yielded these values for the constants: V_{max} = 100, K_m = 9.28 x 10^{-4}

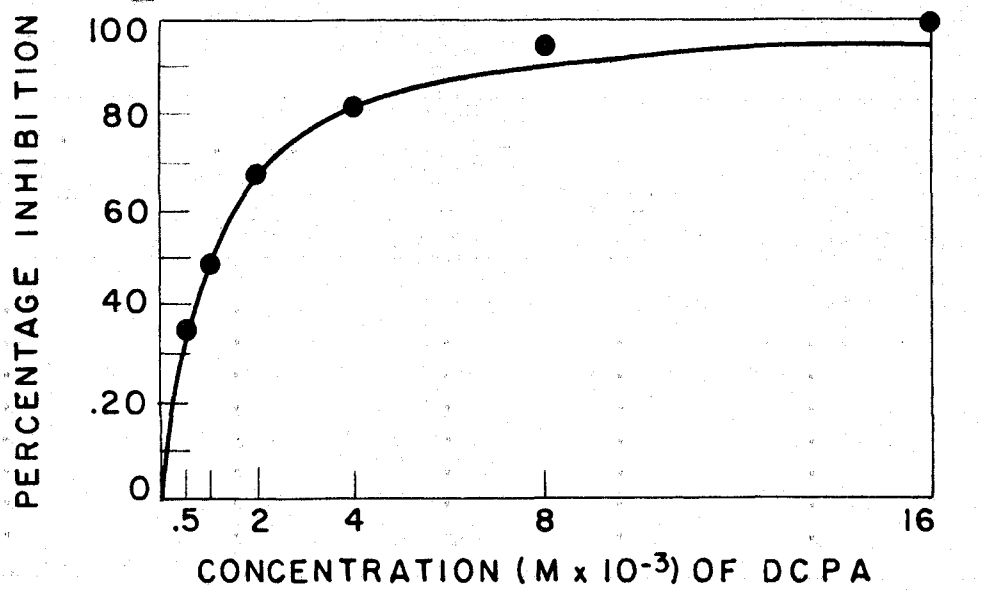

Fig. 5. Inhibition of growth (soybean seedlings) as a function of the concentration of DCPA. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of fresh weights

shown. The same mathematical procedures have been used as described for the preceding experiment. Here again the reciprocal plot of the data shows a very good fit to a straight line, and the calculated and experimental percentage inhibition show good agreement.

Figs. 6 and 7 represent the effect of 2,4,5-T on soybeans. Although there is somewhat more deviation from linearity in the reciprocal plot than encountered with 2,4-D or DCPA, the calculated percentage inhibition agrees closely in form to that shown by the first two compounds. Of further interest is the fact that inhibition was obtained with a lower range of concentrations than were used with 2,4-D. This difference is also shown by comparing the K_m values for the two compounds. The K_m values will be explained in more detail below.

The reciprocal plot for TCA is shown in Fig. 8. This compound is similar in structure to DCPA and is thought to be similar in mode of action. In the light of these facts it is interesting to note that nearly identical K_m values were obtained for both compounds. The variability encountered in this experiment was high, the coefficient of variability being on the order of 46 per cent. In spite of this high degree of variation the reciprocal plot of the data shows a good fit to a straight line.

Amino triazole (AT) is a relatively new herbicide,

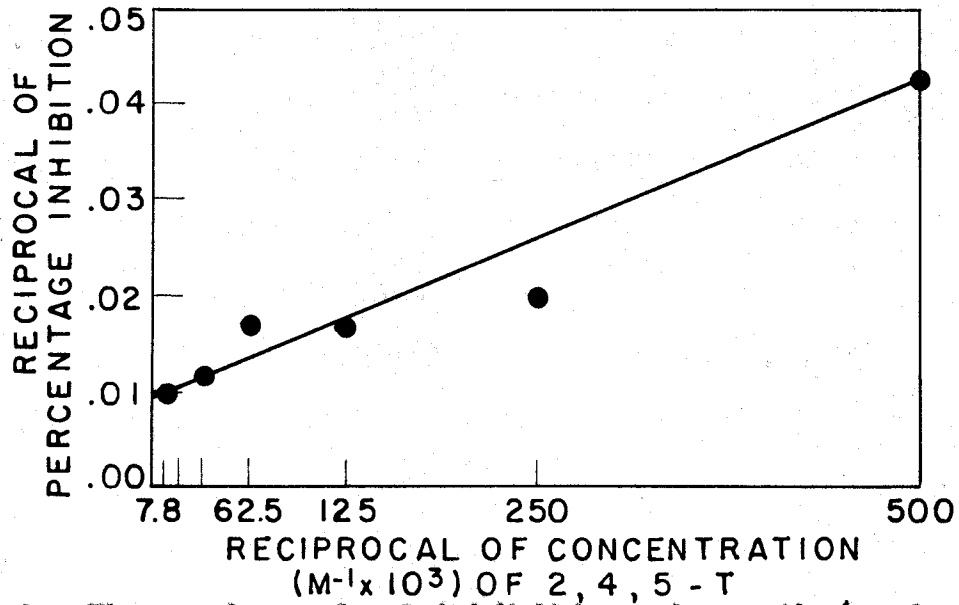

Fig. 6. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of 2,4,5-T. Calculation of linear regression yielded these values for the constants: V_{max} = 100, K_m = 6.22 x 10^{-6}

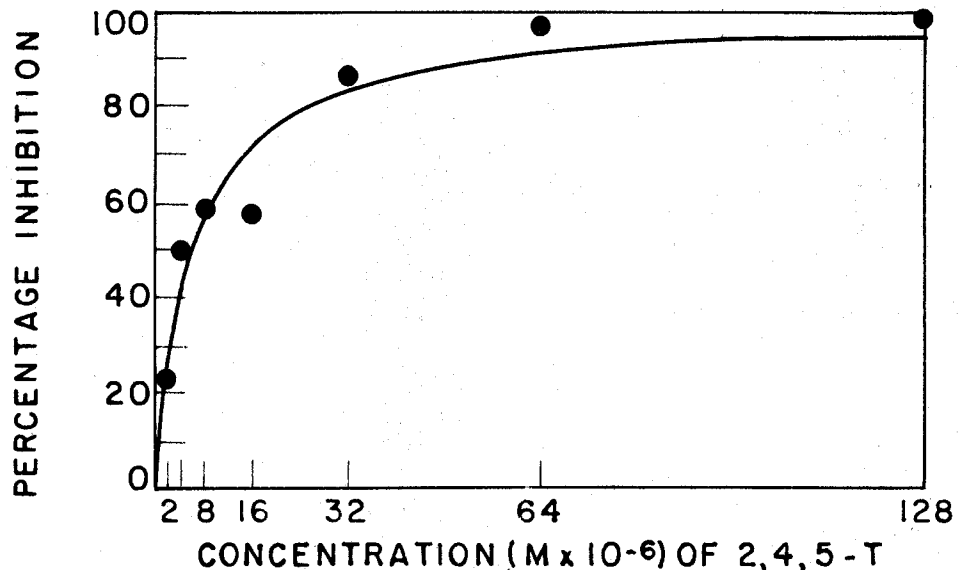

Fig. 7. Inhibition of growth (soybean seedlings) as a function of the concentration of 2,4,5-T. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of fresh weights

systemic in its action, with an ability to disrupt chlorophyll synthesis. The reciprocal plot of the data is shown in Fig. 9. The range of molar concentrations over the inhibitory range was similar to that used for DCPA and TCA, although the K_m value obtained was somewhat larger.

IAA was included in these experiments to compare the response obtained with that of 2,4-D, and also because it is thought to be the native auxin within plants. Although the ability of the two compounds to stimulate plant growth in low concentrations has been reported to be similar, it was found that higher concentrations of IAA than of 2,4-D were needed to inhibit growth to the same extent. It can be seen in Fig. 10 that the maximum velocity of inhibition obtained with IAA was 81 per cent. It was noted during the course of the experiment that the initial formative effects induced by IAA were similar to those induced by 2,4-D. Plants treated with IAA, however, were able to recover from these effects (epinasty, stem-bending, and proliferation) more completely than plants treated with 2,4-D.

Maleic hydrazide (MH) was the final compound used in this series of experiments. This compound occupies an unique position in the herbicidal field in that it inhibits growth without inducing morphological abnormalities. It has also found a place in growth studies because it inhibits auxin action at low concentrations. The reciprocal plot is shown

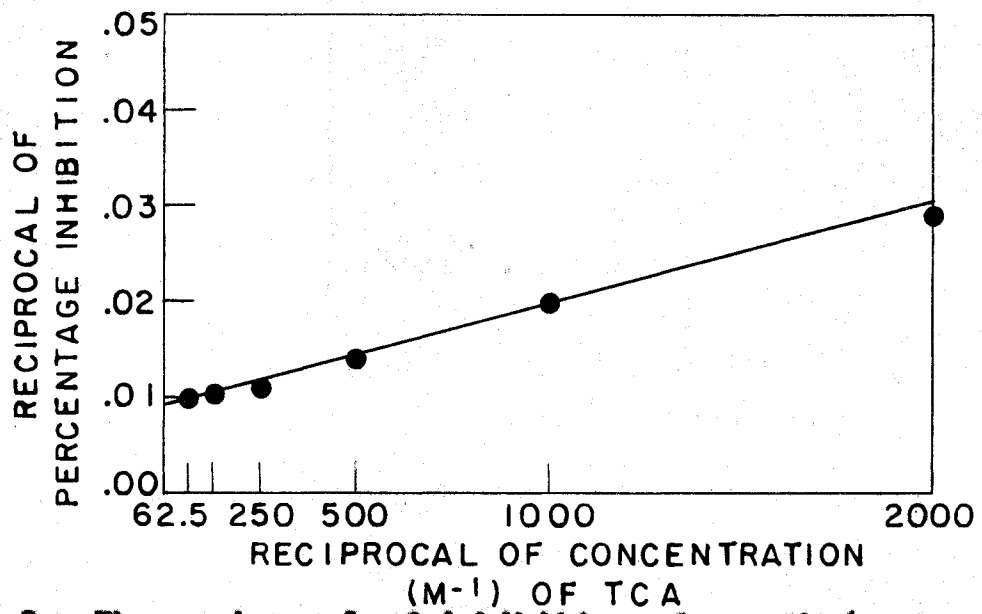

Fig. 8. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of TCA. Calculation of linear regression yielded these values for the constants: $V_{max} = 100$, $K_m = 10^{-3}$

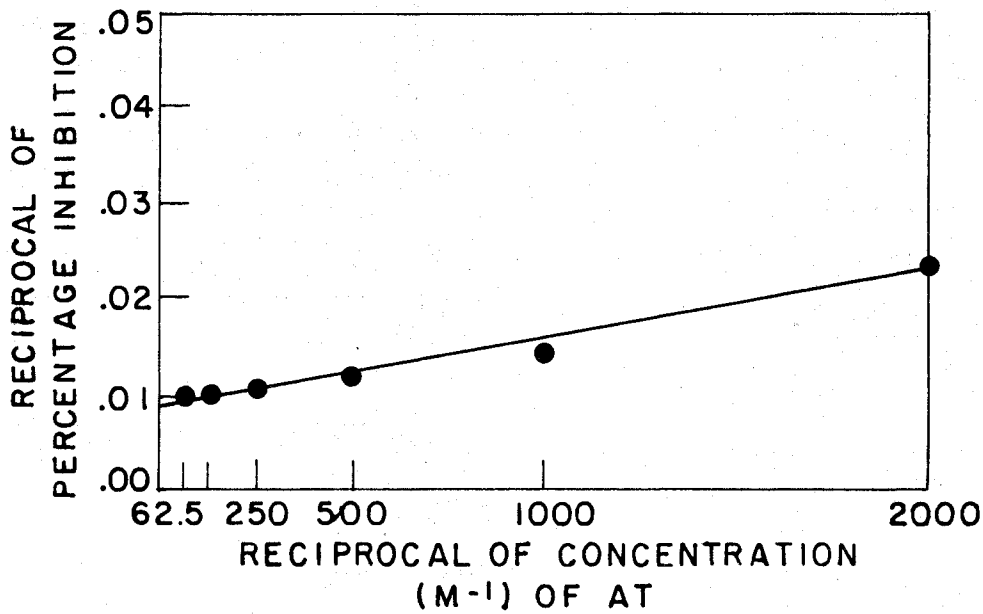

Fig. 9. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of AT. Calculation of linear regression yielded these values for the constants: $V_{max} = 100$, $K_m = 6.94 \times 10^{-4}$

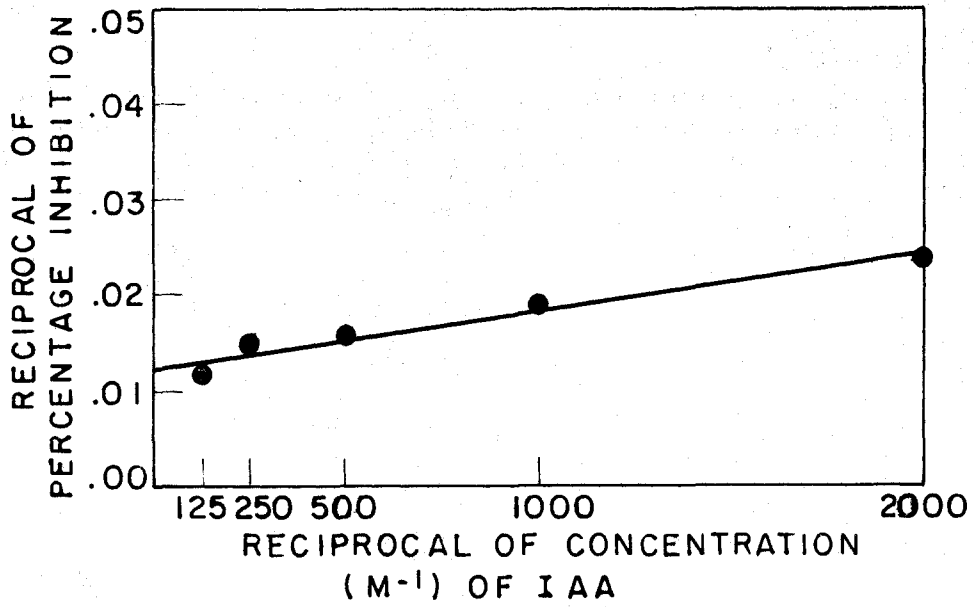

Fig. 10. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of IAA. Calculation of linear regression yielded these values for the constants: V_{max} = 81.3, K_m = 5.04 x 10^{-4}

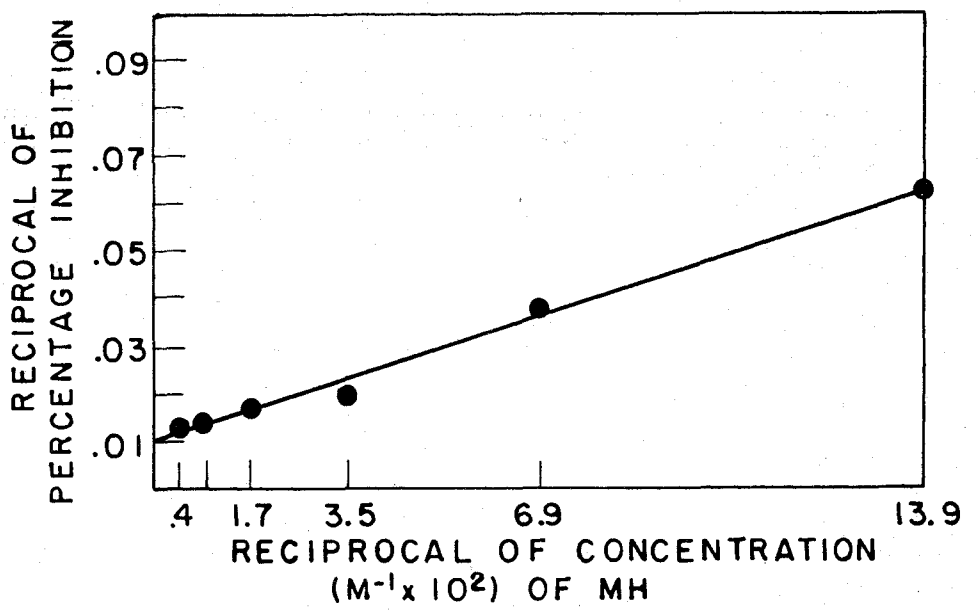

Fig. 11. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of concentration of MH. Calculation of linear regression yielded these values for the constants: V_{max} = 100, K_m = 3.79 x 10^{-3}

in Fig. 11. MH was somewhat less effective as an herbicide than most of the compounds reported here, as shown by the range of concentrations used and the K_m value obtained.

A summary table of experimental and calculated percentage inhibition is presented in Table 3 for the compounds presented in this section.

It will be noted that in all of these experiments, excepting the one with IAA, the value for V_{max} was 100 per cent. In other words, complete inhibition of growth was obtained with these compounds. Since K_m represents the concentration at which one-half the maximum velocity of inhibition is attained, this value for these compounds is a good estimate of the concentration needed to obtain 50 per cent inhibition. The value obtained for V_{max} in the IAA experiment was 81 per cent, which means that the value for K_m is an estimate of the concentration needed to give about 40 per cent inhibition. The K_m value also serves as a measure of the affinity of the compound for the reactive sites within the plant.

The smaller the K_m value is, the greater is the affinity. This relationship is verified by equation 4 from which it can be seen that increasing concentrations of (HM) will result in decreasing values of K_m. Since a generalized mechanism or site is assumed in this approach, the compounds may be ranked according to the K_m values obtained. The

Table 3. Experimental and calculated percentage inhibition (I) of growth of soybeans

Growth substance	Conc.	Expl. % I	Calc. % I
2,4-D	M x 10^{-4}		
	0.32	21.6	21.3
	0.64	32.9	35.2
	1.28	50.3	52.0
	2.56	68.7	68.5
	5.12	87.7	81.3
	10.24	95.2	89.8
	20.48	98.1	94.5
DCPA	M x 10^{-3}		
	0.5	35.4	35.0
	1.0	49.3	51.9
	2.0	67.9	68.3
	4.0	82.8	81.2
	8.0	94.8	89.6
	16.0	99.3	94.5
2,4,5-T	M x 10^{-6}		
	2.0	23.2	24.3
	4.0	50.8	39.1
	8.0	58.8	56.3
	16.0	58.2	72.0
	32.0	86.3	83.7
	64.0	96.9	91.1
	128.0	99.0	95.4
TCA	M x 10^{-3}		
	0.5	34.5	33.3
	1.0	50.4	50.0
	2.0	71.2	66.7
	4.0	90.6	80.0
	8.0	95.0	88.9
	16.0	97.8	94.1

Table 3. (Continued)

Growth substance	Conc.	Expl. % I	Calc. % I
AT	M x 10^{-3}		
	0.25	17.2	26.5
	0.5	42.4	41.9
	1.0	70.0	59.0
	2.0	82.4	74.2
	4.0	95.2	85.2
	8.0	97.1	92.0
	16.0	99.5	95.8
IAA	M x 10^{-3}		
	0.25	48.4	27.0
	0.5	41.1	40.5
	1.0	52.4	54.1
	2.0	63.2	64.9
	4.0	67.4	72.2
	8.0	85.3	76.5
MH	M x 10^{-3}		
	0.72	15.8	16.0
	1.44	26.7	27.5
	2.88	49.7	43.2
	5.76	57.6	60.3
	11.52	70.3	75.2
	23.04	76.4	85.9

following array is arranged in order of decreasing affinities:

	K_m
2,4,5-T	6.22×10^{-6}
2,4-D	1.18×10^{-4}
IAA	5.04×10^{-4}
AT	6.94×10^{-4}
DCPA	9.28×10^{-4}
TCA	1.00×10^{-3}
MH	3.79×10^{-3}

It may be of significance in speculating on the nature of the receptive site, that the three compounds ranking highest according to their affinities are those known to possess auxin properties in low concentrations.

The variation encountered in these greenhouse studies has been noted in individual experiments. The coefficient of variability ranged from 22 to 46 per cent. The range of concentrations selected, however, was such as nearly to cover the range of inhibition. For this reason it is believed that the variation does not markedly detract from the validity of these results.

The results reported here seem to support the hypothesis advanced. Of especial significance is the accurate transformation of the data to the straight line reciprocal plot,

and the good agreement obtained between experimental and calculated values of percentage inhibition.

Experiments with yeast

Measurements of growth made in experiments with yeast were analyzed in the same manner as those of the soybean experiments. The growth attained by the controls was considered to be 100 per cent and was used to obtain the percentage inhibition. The plot of percentage inhibition against concentration resulted in hyperbolas of a somewhat different shape than those obtained with soybeans (Fig. 13). It was generally found that the concentration needed to obtain a given response in yeast was considerably higher than that for soybeans. Also, a narrower range of concentrations covered the complete range of growth inhibition.

Results are presented for 2,4-D in Figs. 12 and 13. The double reciprocal plot of the data shows a good fit to the straight line. It will be noted that the line does not intercept the ordinate at 0.01 (the reciprocal of 100 per cent inhibition). The intercept calculated from linear regression was 0.0041 which corresponds to a V_{max} of 244 per cent. This value has no physiological interpretation since the maximum velocity can be no greater than 100 per cent. The value obtained for K_m is also correspondingly high. In terms of concentration, it is of greater magnitude than the

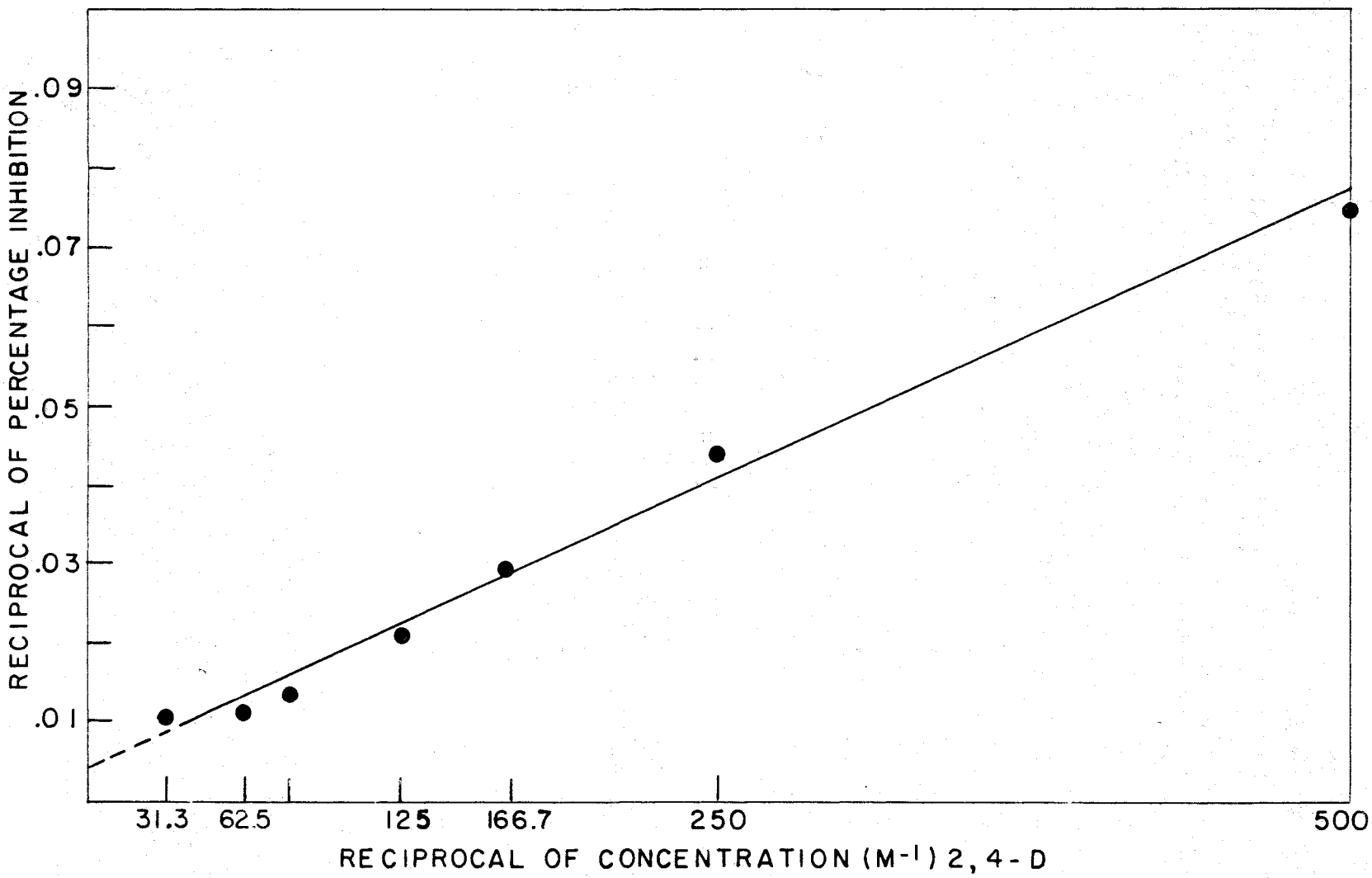

Fig. 12. The reciprocal of inhibition of yeast growth as a function of the reciprocal of concentration of 2,4-D. Calculation of linear regression yielded these values for the constants: $V_{max} = 244$, $K_m = 3.54 \times 10^{-2}$

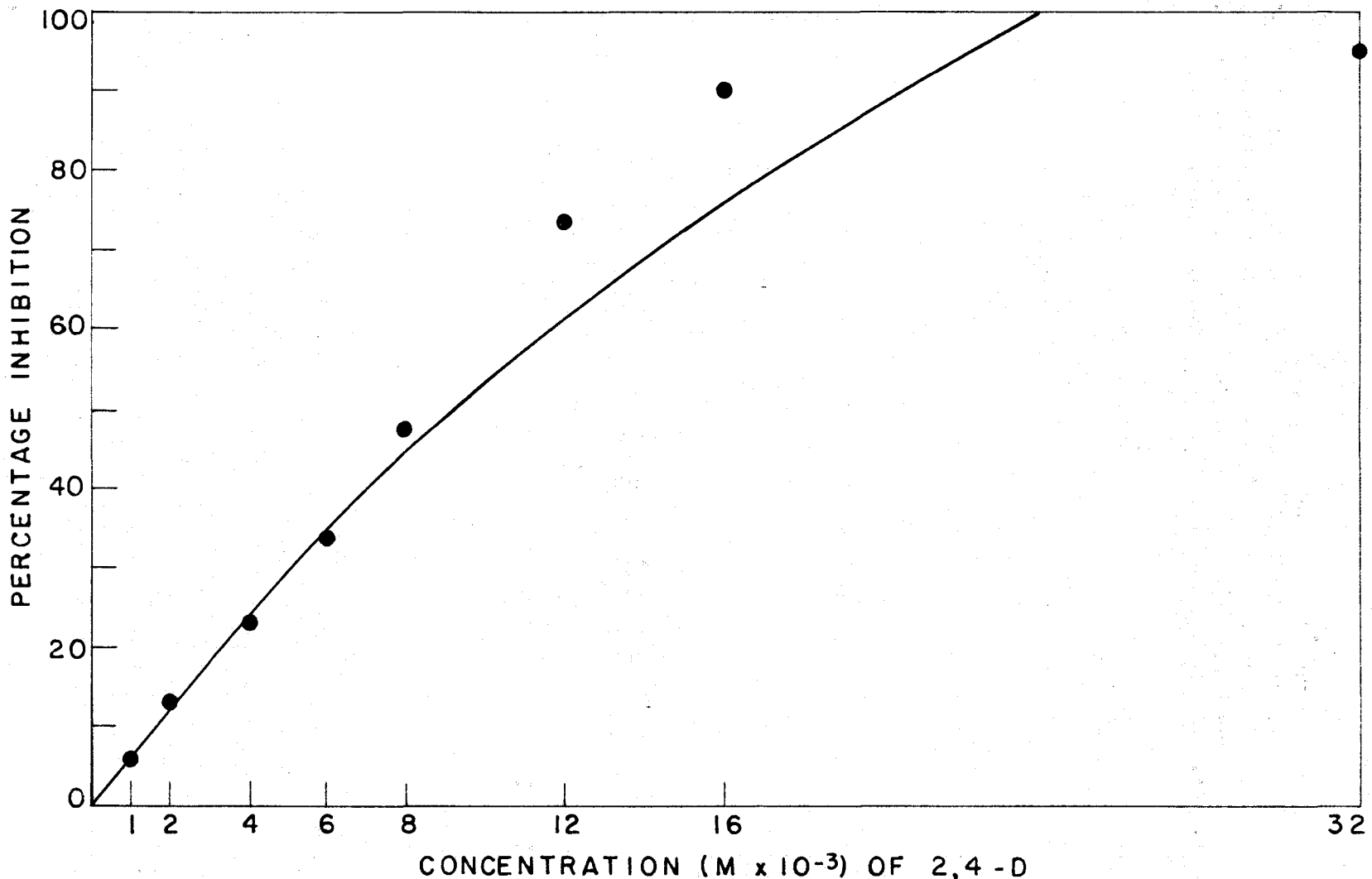

Fig. 13. Inhibition of yeast growth as a function of the concentration of 2,4-D. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of turbidity of cultures

highest concentration used in this experiment. Of perhaps greater significance than the calculated constants is the point where the reciprocal line crosses the abscissa at a V_{max} of 100 per cent. This may be considered to be the concentration at which maximum velocity is reached and it corresponds to a value of 0.027 M. It is also possible to obtain a graphical representation of the concentration at which one-half maximum velocity (50 per cent inhibition) occurs. This would be the reciprocal concentration at the point where the line crosses the abscissa of the reciprocal inhibition of 0.02 and corresponds to a value of 9.14×10^{-3}. Since purely mathematical procedures were used in fitting the line to the experimental points and in calculating the original constants, these same constants must be used in obtaining the calculated percentage inhibition. These calculated values are shown by the continuous line in Fig. 13. Good agreement is shown at low concentrations but some divergence is noted at higher concentrations. The concentration at which the curve reaches 100 per cent corresponds to the concentration in the reciprocal plot at which a maximum velocity of 100 per cent is reached. The continuation of the curve above 100 per cent, again would have no physiological interpretation.

Coumarin has been included in these studies because of its reported similarity to 2,4-D in growth inhibition studies

(7, 9, 17). It was found to inhibit growth at a somewhat lower range of concentration than did 2,4-D. The straight line and hyperbolic plots are shown in Figs. 14 and 15. The same general tendencies are noted here as were seen in the 2,4-D experiment; i. e., the maximum velocity of 100 per cent is reached by the reaction before the line intercepts the ordinate. The calculated intercept value, therefore, has no physiological meaning. The fit of the straight line to the points is again good, however, and the agreement between calculated and experimental percentage inhibition is very close (Fig. 15).

The results for IAA are shown in Figs. 16 and 17. The range of concentrations used was identical to those used for 2,4-D. The maximum velocity attained, however, was considerably less, being on the order of 70 per cent. This result is in agreement with the result obtained with IAA on soybeans, although the concentrations needed to obtain inhibition of growth of yeast were greater. It will be noted that the calculated constants take on a physiological importance not obtained with 2,4-D and coumarin, principally because complete inhibition of growth was not reached, even at higher concentrations. The calculated maximum velocity is in good agreement with the level of inhibition attained, and the value for $\underline{K_m}$ is a good estimate of the concentration needed to attain one-half $\underline{V_{max}}$. The agreement obtained with IAA

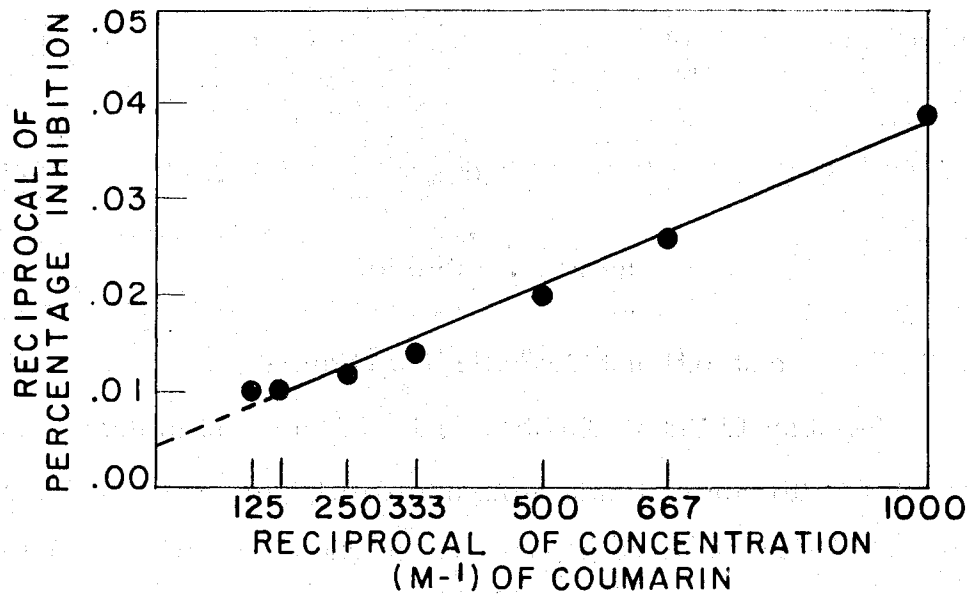

Fig. 14. The reciprocal of inhibition of yeast growth as a function of the reciprocal of concentration of coumarin. Calculation of linear regression yielded these values for the constants: $V_{max} = 217$, $K_m = 7.07 \times 10^{-3}$

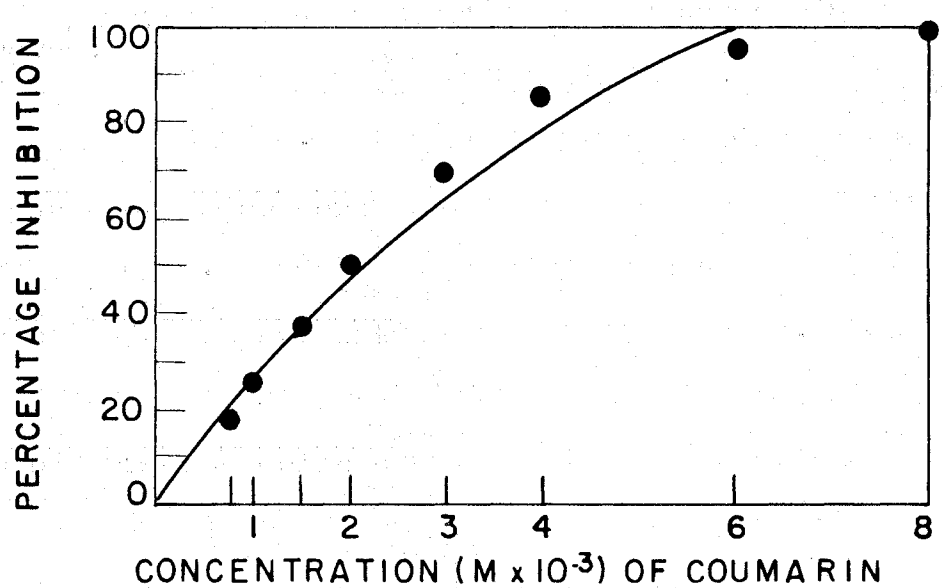

Fig. 15. Inhibition of yeast growth as a function of the concentration of coumarin. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of turbidity of cultures

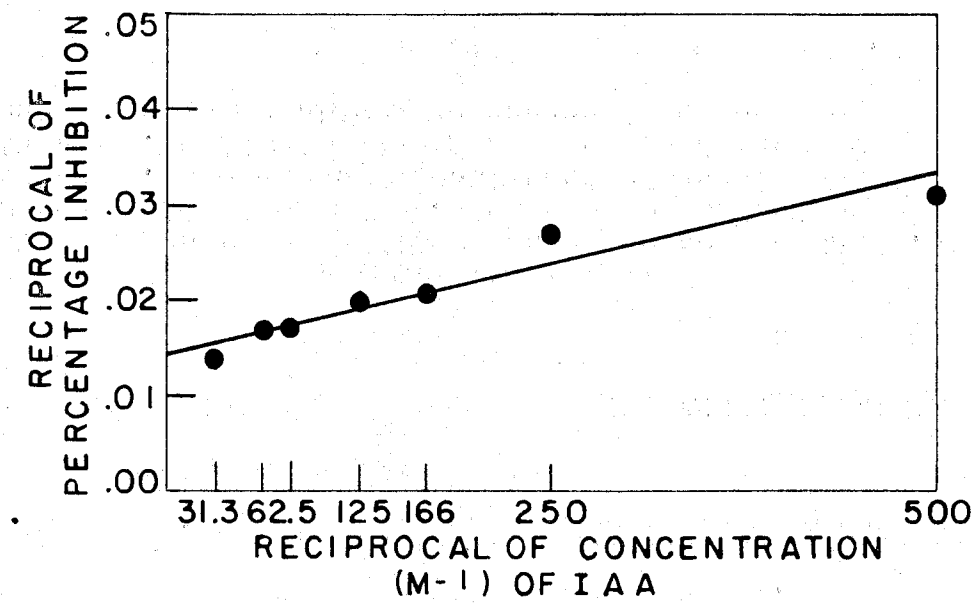

Fig. 16. The reciprocal of inhibition of yeast growth as a function of the reciprocal of concentration of IAA. Calculation of linear regression yielded these values for the constants: V_{max} = 69, K_m = 2.55 x 10^{-3}

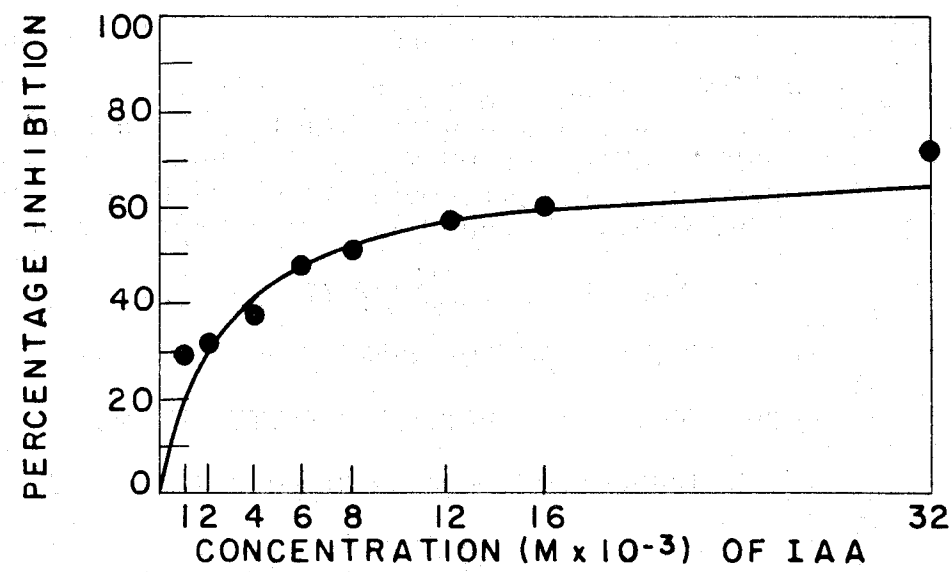

Fig. 17. Inhibition of yeast growth as a function of the concentration of IAA. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of turbidity of cultures

between such widely different plants as soybeans and yeast is of considerable interest, especially since IAA is considered by many to be the native growth hormone of plants.

The last compound to be reported in this section is 2,4,5-T. The graphical results are presented in Figs. 18 and 19. The concentrations needed to cover the inhibitory range were somewhat less than those required for the other three compounds, and the range from highest to lowest was considerably narrower. The reciprocal plot of the data does not show as good a fit to the calculated regression line as was obtained with the three compounds reported above. Considerable divergence occurred also between calculated and experimental percentage inhibition, especially at higher concentrations.

A summary table of experimental and calculated percentage inhibitions is presented in Table 4.

Results obtained from these experiments with yeast are not so clear cut as those from the soybean experiments. Of particular difficulty is the exact interpretation of the values obtained for the constants. As stated previously, values greater than 100 per cent for the maximum velocity can have no physiological meaning. Likewise, the exact meaning to be assigned to the $\underline{K_m}$ values in these experiments is not clear. Although these values are of little use in estimating one-half $\underline{V_{max}}$ (with the exception of IAA), they

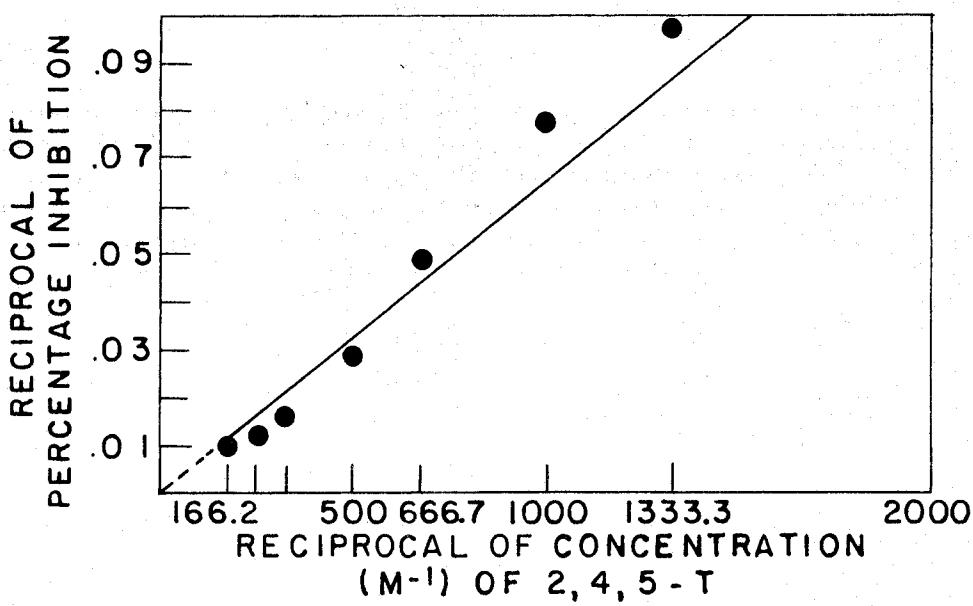

Fig. 18. The reciprocal of inhibition of yeast growth as a function of the reciprocal of concentration of 2,4,5-T. Calculation of linear regression yielded these values for the constants: $V_{max} = 1000$, $K_m = 6.40 \times 10^{-2}$

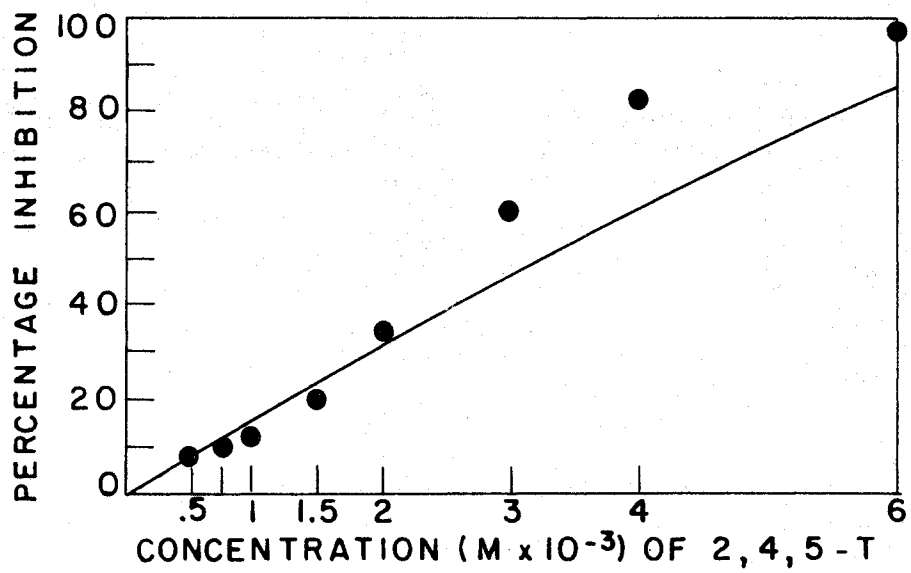

Fig. 19. Inhibition of yeast growth as a function of the concentration of 2,4,5-T. The solid line represents the hyperbola described by equation 6. The points represent experimental determinations. Growth was measured by increase of turbidity of cultures

Table 4. Experimental and calculated percentage inhibition (I) of growth of yeast

Growth substance	Conc. $\underline{M} \times 10^{-3}$	Expl. % I	Calc. % I
2,4-D	1.0	5.8	6.7
	2.0	13.3	13.1
	4.0	22.7	24.8
	6.0	34.0	35.3
	8.0	47.5	45.0
	12.0	73.5	61.7
	16.0	90.1	75.8
	32.0	95.2	
Coumarin	0.75	19.2	20.8
	1.0	25.7	26.9
	1.5	37.9	38.0
	2.0	50.1	47.8
	3.0	69.4	64.5
	4.0	84.9	78.1
	6.0	96.4	99.8
	8.0	99.7	
IAA	1.0	29.1	19.4
	2.0	31.8	30.3
	4.0	37.3	41.2
	6.0	48.5	48.4
	8.0	51.0	52.3
	12.0	57.7	56.9
	16.0	60.3	59.5
	32.0	72.6	64.3
2,4,5-T	0.5	8.6	7.8
	0.75	10.3	11.6
	1.0	12.8	15.4
	1.5	20.4	22.9
	2.0	34.6	30.3
	3.0	60.5	44.8
	4.0	83.3	58.8
	6.0	97.9	85.7

may be of value in determining the relative inhibitory efficiencies of the compounds. The array of compounds in order of increasing K_m values (decreasing inhibitory efficiency) is as follows:

	K_m
IAA	2.55×10^{-3}
Coumarin	7.07×10^{-3}
2,4-D	3.54×10^{-2}
2,4,5-T	6.4×10^{-2}

It is interesting to note that the two compounds which showed the greatest lack of conformity in this analysis rank the lowest in affinity for inhibition mechanisms or sites in terms of their K_m values.

The K_m' values which correspond to one-half the maximum velocity of 100 per cent (or 50 per cent inhibition) were read directly from the double reciprocal plots. The three compounds may then be ranked according to these values obtained in order of decreasing affinities as follows:

	K_m'
Coumarin	2.13×10^{-3}
2,4,5-T	3.50×10^{-3}
2,4-D	9.14×10^{-3}

The values thus obtained for coumarin and 2,4-D are in close

agreement with the actual concentrations giving approximately 50 per cent inhibition, which is an indication of the close fit of the reciprocal line to the experimental points. The value obtained for 2,4,5-T, however, is somewhat higher than the range of concentrations giving approximately 50 per cent inhibition, primarily because of the discrepancy between the reciprocal line and the experimental points.

It is also of interest to note the concentrations at which a maximum velocity of 100 per cent was reached. These values also were read directly from the double reciprocal plots and are as follows:

Coumarin	6×10^{-3}
2,4,5-T	6×10^{-3}
2,4-D	2.7×10^{-2}

Mixtures of Compounds

Experiments with soybeans

The kinetic analysis of growth inhibition affords a means of evaluating quantitatively the type of response obtained when the inhibition induced by an active compound is modified by the presence of a second compound, which may or may not be active in producing growth inhibition. In the simplest type of action, two such compounds may be specific in their requirements for the same mechanism or receptive

site in the plant which ultimately gives rise to growth inhibition. In this type of action the molecules of the two compounds compete with one another for these sites, the concentrations of which are considered to be limited relative to the concentrations of the competing growth inhibitors. These relationships may be expressed in analogy with Lineweaver and Burk (52) by the following equation:

$$H + H' + 2M \rightleftharpoons HM + H'M \longrightarrow \text{Inhibition of growth} \qquad (9)$$

in which part of the site (M) originally available to the primary active compound (H) is now combined with the secondary compound (H') which is relatively less active than the former. The initial velocity equation may be written as follows:

$$v = \frac{V_{max}(H)K_m'}{K_m \cdot K_m' + K_m(H') + K_m'(H)} \qquad (10)$$

The maximum velocity of the reaction will be attained only when the concentration of $\underline{H}$ greatly exceeds the concentration of $\underline{H'}$ and all of the mechanism or sites (M) will be in combination with $\underline{H}$ and none in the form of $\underline{H'M}$.

The reciprocal of equation 10 is written as follows:

$$\frac{1}{v} = \frac{1}{V_{max}} \left[K_m + \frac{K_m(H')}{K_m'} \right] \frac{1}{(H)} + \frac{1}{V_{max}} \qquad (11)$$

It is apparent from this equation that a plot of $\frac{1}{v}$ against $\frac{1}{(H)}$ will result in a line, the slope of which is increased over that expressed by equation 6, by the factor $1 + \frac{(H')}{K_m'}$, while the intercept $(\frac{1}{V_{max}})$ remains unchanged. Competition between the two compounds, therefore, is more apparent at low concentrations of the primary compound and is largely overcome in favor of the primary compound by increasing concentrations of H. The competition will result in a lesser inhibition at low concentrations of H than would be shown by the primary compound alone.

This type of response is shown in Table 5 and Fig. 20. In this experiment various concentrations of 2,4-D and 2,4,5-T were applied singly and in combination to soybean seedlings. The primary compound is considered to be 2,4-D

Table 5. Percentage inhibition of growth (soybean seedlings) as a function of concentrations of 2,4-D and 2,4,5-T applied singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of 2,4,5-T			
	None	2.5×10^{-7}	10^{-6}	4×10^{-6}
None		36.5	34.9	43.6
3.2×10^{-5}	57.3	50.6	41.9	47.7
6.4×10^{-5}	65.6	66.0	50.2	60.6
12.8×10^{-5}	78.4	71.4	62.2	77.2
25.6×10^{-5}	85.9	78.8	84.6	86.7

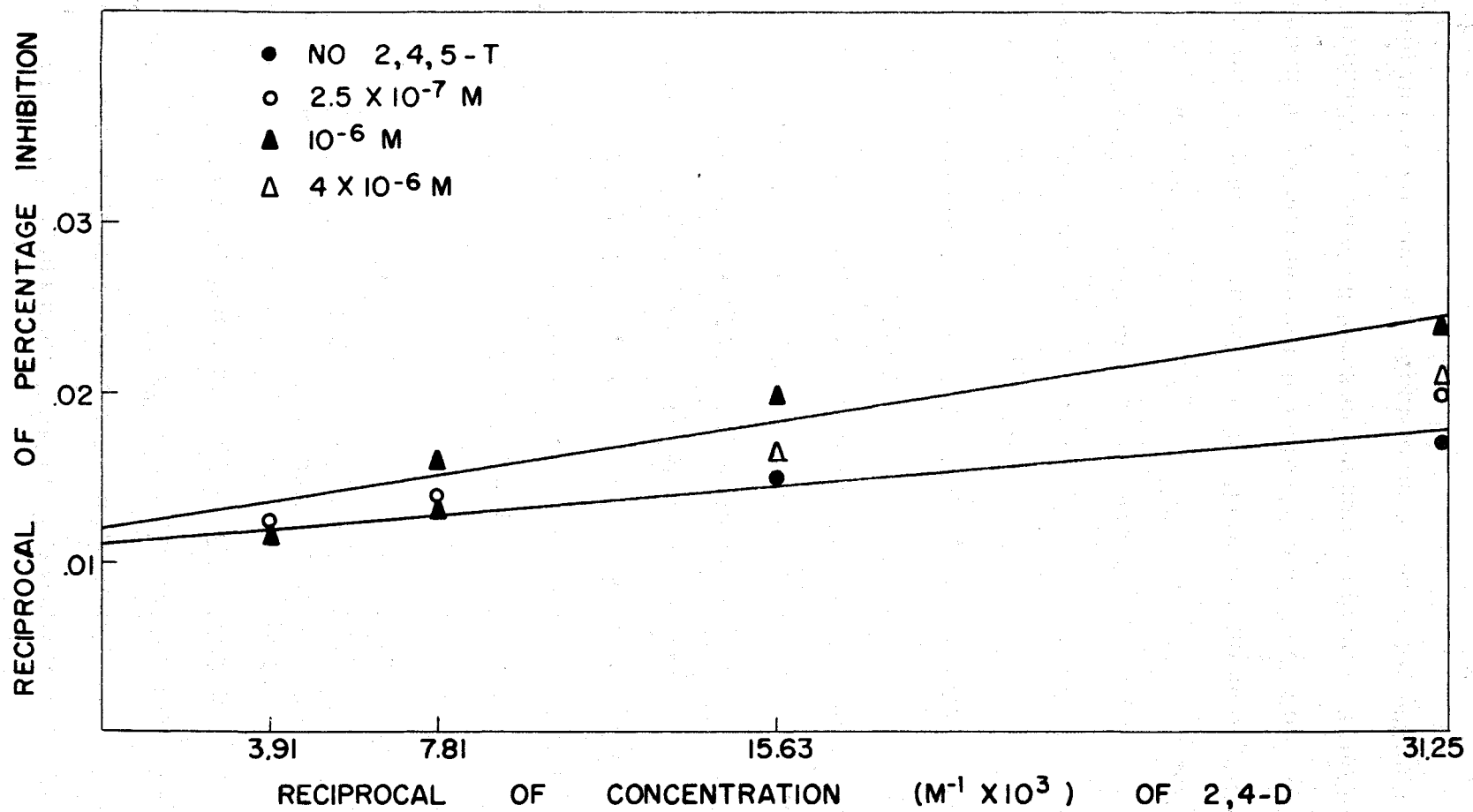

Fig. 20. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of the concentration of 2,4-D at four levels of 2,4,5-T

and 2,4,5-T is the competitor. In Fig. 20 the reciprocal of the percentage inhibition is plotted against the reciprocal of the concentration of 2,4-D for each of the four levels of 2,4,5-T. To avoid confusion, only two of the four lines are shown in the graph. The lower line is the double reciprocal plot of 2,4-D with no 2,4,5-T added. The slopes of the other three lines are all greater. Although small differences were encountered in the intercepts on the ordinate, it is probable that the differences are within the range of variability. Therefore, a common $\underline{V_{max}}$ is assumed for the four reactions. Calculated t-values for differences between slopes of the lines show that no acceptable level of significance exists between the zero and low level of 2,4,5-T. Slopes of the two lines representing the intermediate and high concentrations of 2,4,5-T show acceptable levels of significant differences from the slope of the line representing the zero level at probability levels of approximately 90 per cent for the intermediate concentration and 95 per cent for the high concentration. Although the upper line in the graph represents the intermediate concentration, no significant difference exists between the slope of this line and the slope of the line representing the high concentration of 2,4,5-T.

The results obtained with mixtures of IAA and 2,4-D are shown in Table 6 and Fig. 21. Considerable deviation from

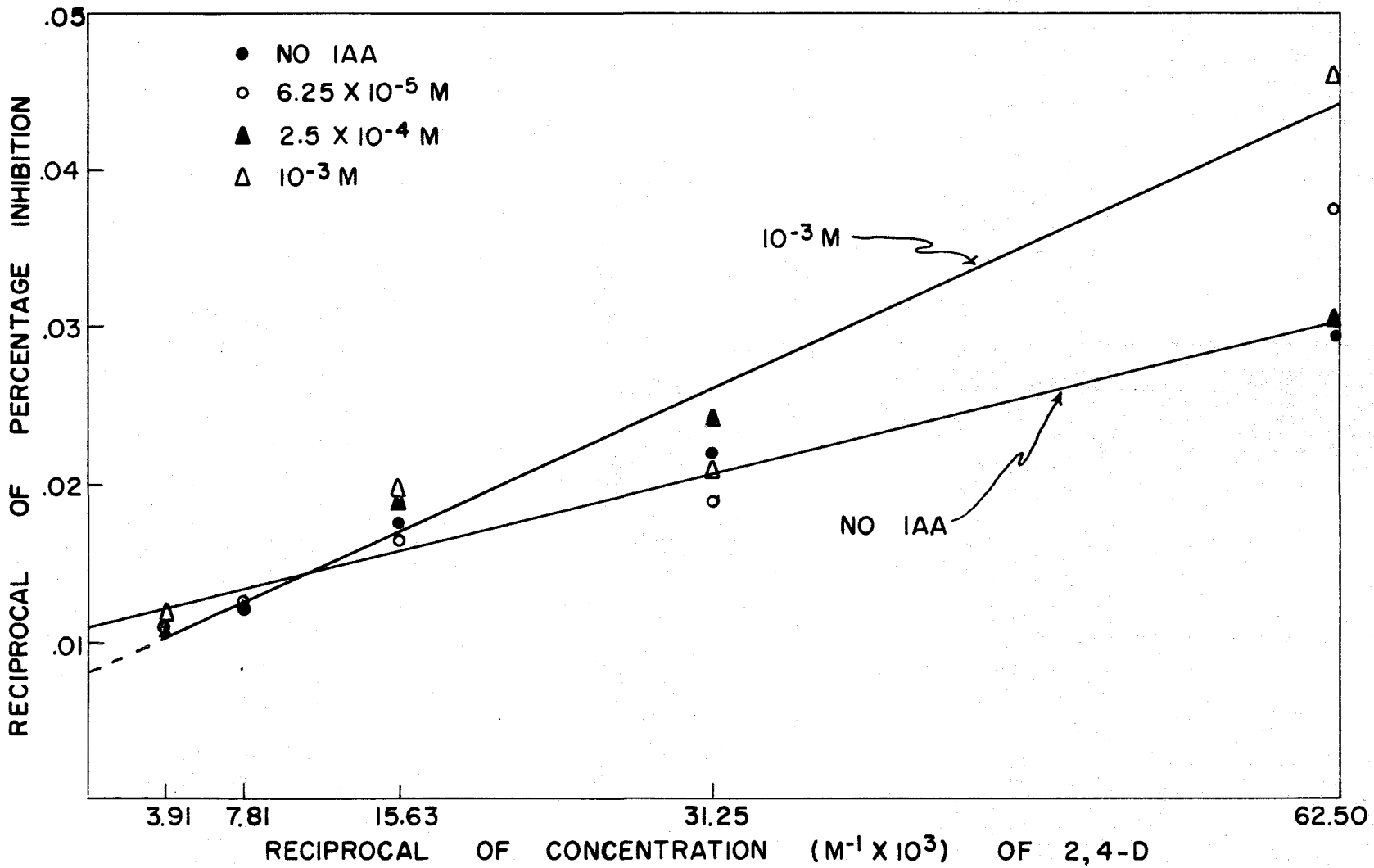

Fig. 21. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of the concentration of 2,4-D at four levels of IAA

Table 6. Percentage inhibition of growth (soybean seedlings) as a function of concentrations of 2,4-D and IAA applied singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of IAA			
	None	6.25×10^{-5}	2.5×10^{-4}	10^{-3}
None		5.8	9.3	13.2
1.6×10^{-5}	33.8	26.7	32.9	21.7
3.2×10^{-5}	45.3	53.1	41.5	48.1
6.4×10^{-5}	56.6	60.1	53.1	50.8
12.8×10^{-5}	82.2	80.2	81.4	80.6
25.6×10^{-5}	91.1	91.5	92.6	88.8

the regression lines was noted, as shown by the experimental points in the graph. Again, only two of the four lines are shown, the upper one representing the double reciprocal plot of inhibition by 2,4-D as modified by the high (10^{-3}M) level of IAA, and the lower line corresponding to the zero level of IAA. Due to the variation shown in the experiment, no particular importance is attached to the crossing of the lines or the discrepancy between the intercepts. Assuming, therefore, that the four reactions share a common maximum velocity, it becomes permissible to compare the slopes of the lines to determine whether or not competition exists

between the two compounds. That such competition does exist is indicated by the calculated t-values, from which it was determined that the slopes of the lines representing the low and high levels of IAA differed significantly from the zero-level line at probability levels of 90 and 95 per cent, respectively. The erratic behavior of applied IAA noted in these experiments may account for the apparent change of positions of the low and intermediate levels of IAA. The line representing the intermediate level of IAA was not significantly different from the line representing the zero level. Of particular importance in this experiment is the fact, shown in Table 6, that IAA applied alone produced a low level of growth inhibition at all three concentrations. IAA, therefore, may be considered relatively inactive in the mixture as compared to 2,4-D. This fact serves to strengthen the hypothesis that IAA competitively inhibits the action of 2,4-D at low concentrations, an effect which is overcome by additions of higher concentrations of 2,4-D.

Another type of response is that of two compounds in a mixture acting together in such a way as to bring about increased inhibition at low concentrations of the primary compound. This type of action may be due to the mutual sharing of a receptive site or to separate action on separate sites, both of which lead to growth inhibition. A response of this type is shown by the effect of mixtures of TIBA and 2,4-D on

soybeans (Table 7 and Fig. 22). TIBA has been shown to interact with auxin at low concentrations to increase the stimulation of growth (3, 32, 85, 91). In this experiment TIBA alone inhibited growth (Table 7), and, in combinations, enhanced the action of 2,4-D. This effect is shown in the double reciprocal plots by the decrease in the slopes of the lines below that of the line representing the action of 2,4-D alone. According to the calculated t-values there is no significant difference between the slopes of the lines representing the zero and low levels of TIBA. The slopes of the lines representing the intermediate and high levels of TIBA are significantly different from the zero level at probability

Table 7. Percentage inhibition of growth (soybean seedlings) as a function of concentrations of 2,4-D and TIBA applied singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of TIBA			
	None	2.5×10^{-6}	10^{-5}	4×10^{-5}
None	—	11.8	16.5	40.2
3.2×10^{-5}	28.9	28.2	40.9	49.4
6.4×10^{-5}	49.0	41.2	62.0	58.7
12.8×10^{-5}	75.8	66.5	79.1	82.9
25.6×10^{-5}	80.1	80.9	84.9	91.5

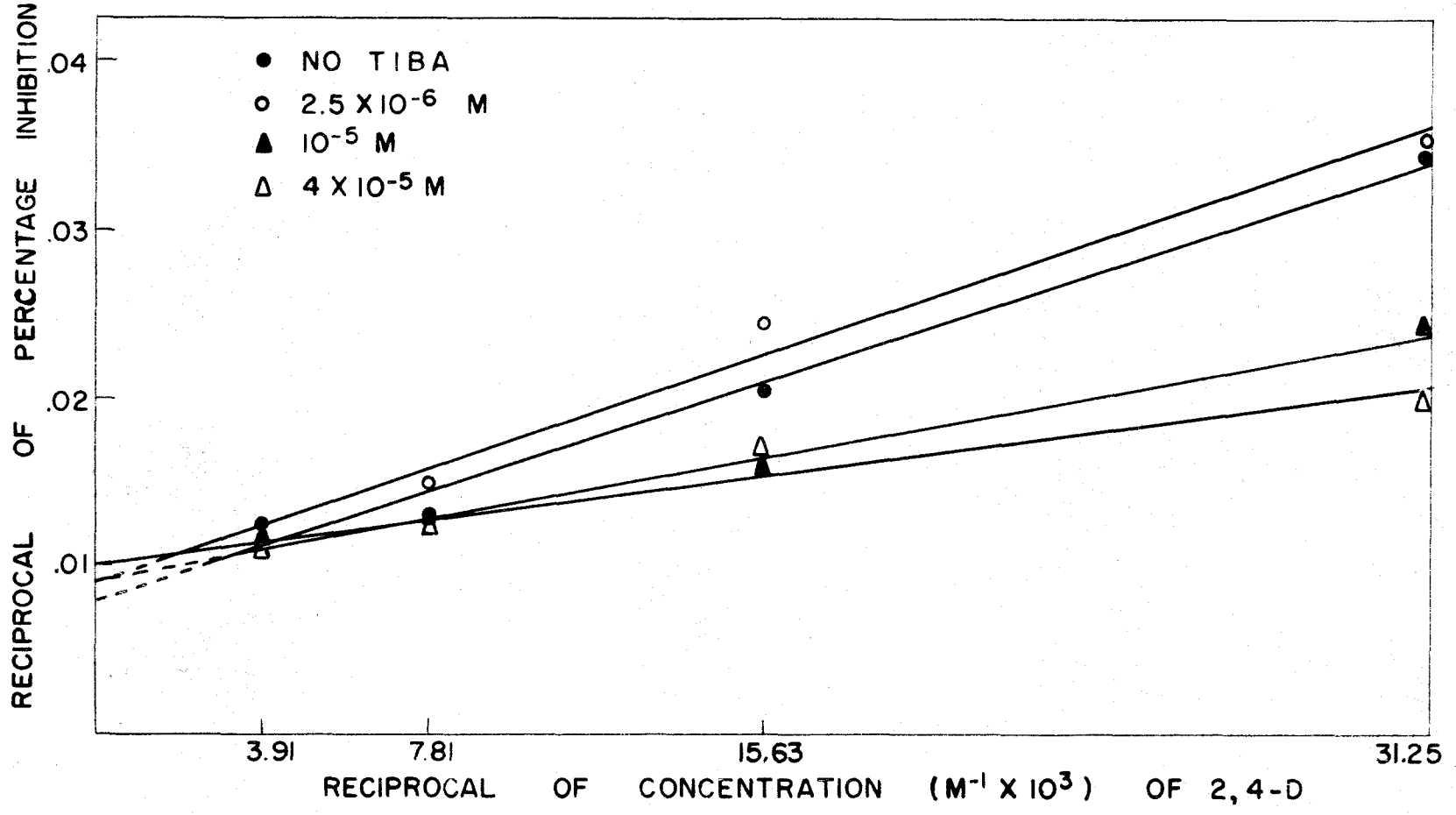

Fig. 22. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of the concentration of 2,4-D at four levels of TIBA

values exceeding 95 per cent. The lines for the two high levels of TIBA differ from one another at a probability level of approximately 90 per cent. The intercepts of the lines on the ordinate are again assumed to be nearly identical.

Mixtures of maleic hydrazide and 2,4-D produced responses similar to those obtained in the preceding experiment. Again, because of the closeness of three of the four lines, only the lines representing the zero and high level of MH are shown in Fig. 23. The percentage inhibition figures are given in Table 8. Of particular interest in this table is the fact that the two low levels of MH alone produced rather insignificant percentages of inhibition. It

Table 8. Percentage inhibition of growth (soybean seedlings) as a function of concentrations of 2,4-D and MH applied singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of MH			
	None	8×10^{-5}	3.2×10^{-4}	12.8×10^{-4}
None		2.5	7.9	65.0
3.2×10^{-5}	37.9	42.9	43.8	66.0
6.4×10^{-5}	64.0	74.9	61.6	75.9
12.8×10^{-5}	87.7	89.2	84.7	91.6
25.6×10^{-5}	96.6	93.1	95.6	95.6

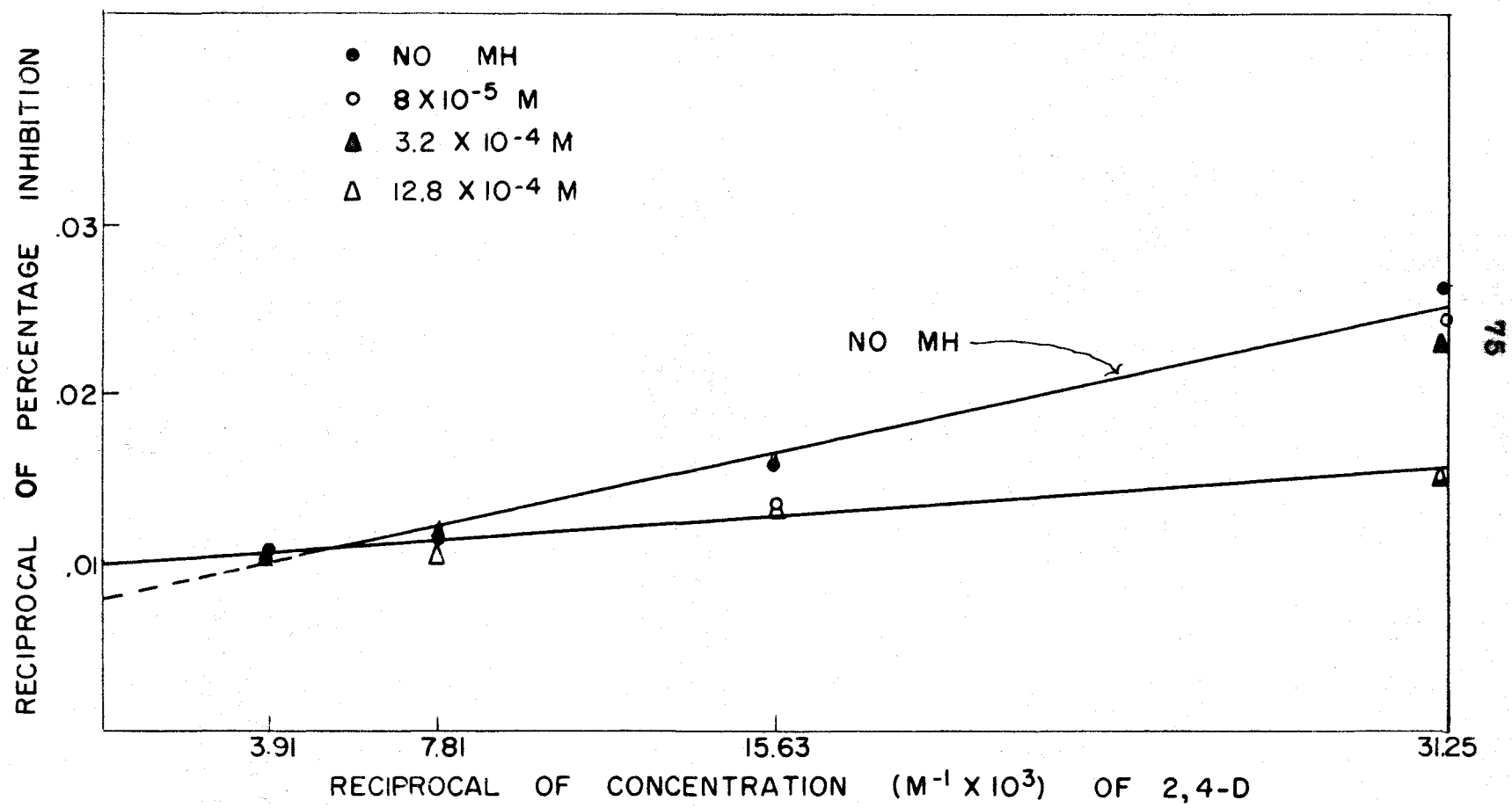

Fig. 23. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of the concentration of 2,4-D at four levels of MH

was found from the calculated t-values for the regression lines that the slope of the line representing the intermediate concentration of MH differed significantly from the slope of the line representing the zero level (probability = 95%), although the low level and the zero level did not differ. The slope of the line representing the high level of MH also differed significantly from the zero-level line (probability = 99%).

The effect of maleic hydrazide in combination with IAA was broadly similar to its action with 2,4-D. The results are presented in Table 9 and Fig. 24. Perhaps the most striking feature to be noted in this experiment is the extreme variability, which seems to be characteristic of IAA applied

Table 9. Percentage inhibition of growth (soybean seedlings) as a function of concentrations of IAA and MH applied singly and in combination

Concentration (M) of IAA	Concentration (M) of MH			
	None	8×10^{-5}	3.2×10^{-4}	12.8×10^{-4}
None		8.9	4.9	35.2
1×10^{-3}	19.3	14.6	22.7	52.0
2×10^{-3}	20.0	27.0	22.3	50.5
4×10^{-3}	35.2	27.6	37.2	58.4
8×10^{-3}	51.6	49.0	59.4	68.6

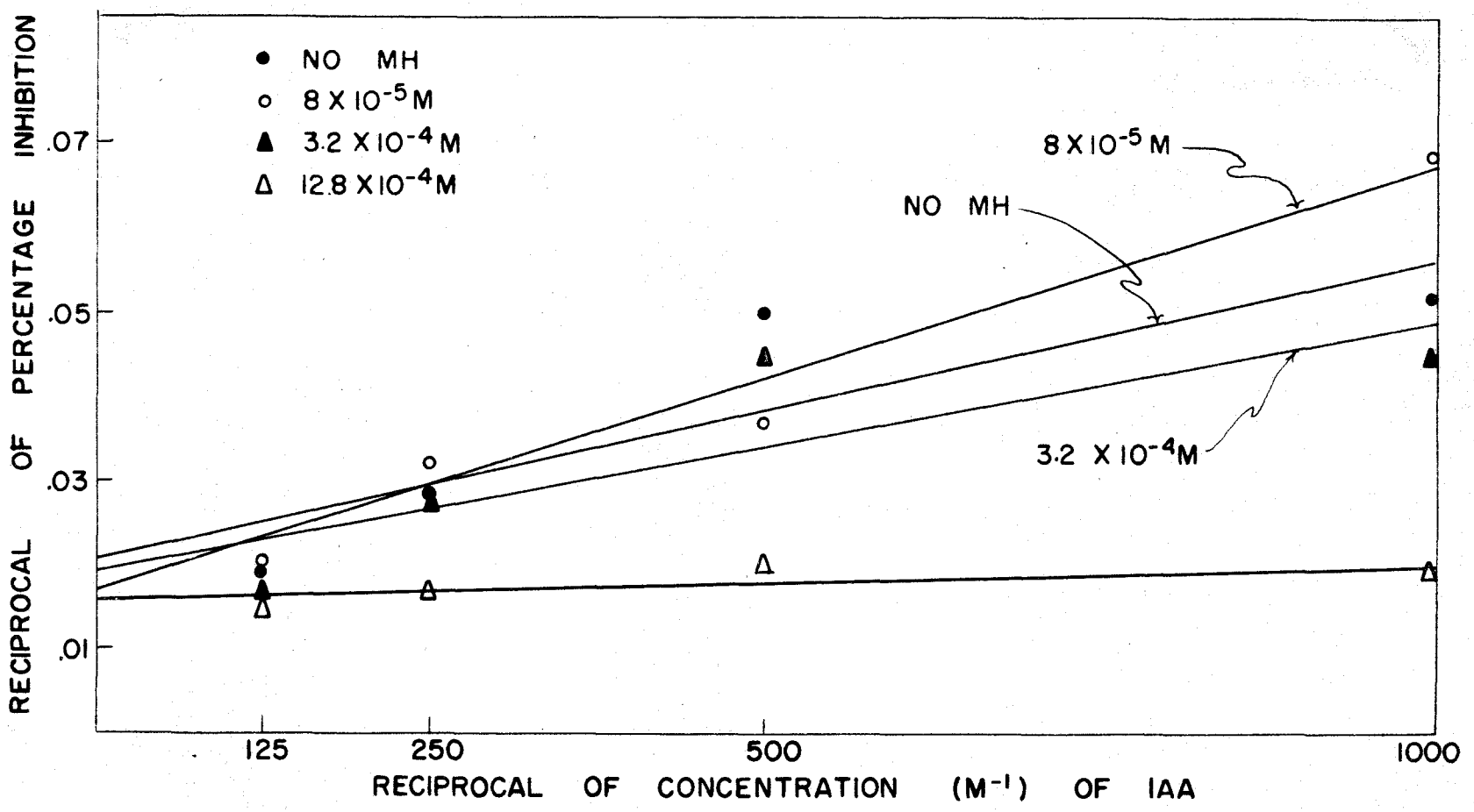

Fig. 24. The reciprocal of inhibition of growth (soybean seedlings) as a function of the reciprocal of the concentration of IAA at four levels of MH

at concentrations in the inhibitory range. Because of this variability, probably, no significant differences exist between the slope of the line representing the zero level of MH and the slopes of the lines for the low and intermediate concentrations of this chemical. The slope of the line for the high level of MH differs from the zero line at a probability value of approximately 90 per cent.

Although the effect of variability cannot be discounted, the results obtained in these experiments indicate that the kinetic analysis can be validly applied to characterize plant growth inhibition induced by mixtures of compounds.

Experiments with yeast

Data from studies of inhibition of growth of yeast in culture media by mixtures of growth substances were analyzed according to procedures described in the preceding section. Only a limited number of flasks could be processed in a short period of time, 15 hours after inoculation. Therefore, only three levels of the secondary or competing chemical were used instead of four as in the soybean experiments. Although good agreement was found between duplicate flasks for one treatment, the double reciprocal plots of the data showed somewhat more deviation from the straight line than was observed in the soybean experiments. It is again assumed, in the four experiments reported here, that a common maximum

velocity is reached for the three levels of the secondary compound.

The results obtained with mixtures of 2,4-D and 2,4,5-T are presented in Table 10 and Fig. 25. It can be seen from the graph that the line representing the low concentration of 2,4,5-T has a greater slope than the line representing 2,4-D alone, which might be an indication of competition between the two compounds. The difference in the slopes of the lines, however, is significant only at a probability level of 80 per cent. Furthermore, it will be noted that the slope of the zero-level line is pulled down considerably by the "falling off" of the last point, which was not noted in the experiment with 2,4-D alone (Fig. 12). Otherwise, the

Table 10. Percentage inhibition of yeast growth as a function of concentrations of 2,4-D and 2,4,5-T added to culture media singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of 2,4,5-T		
	None	5×10^{-4}	2×10^{-3}
None		4.2	34.4
2×10^{-3}	23.7	18.1	49.0
4×10^{-3}	28.5	33.5	64.4
8×10^{-3}	55.5	60.2	87.2
1.6×10^{-2}	98.2	92.9	98.2

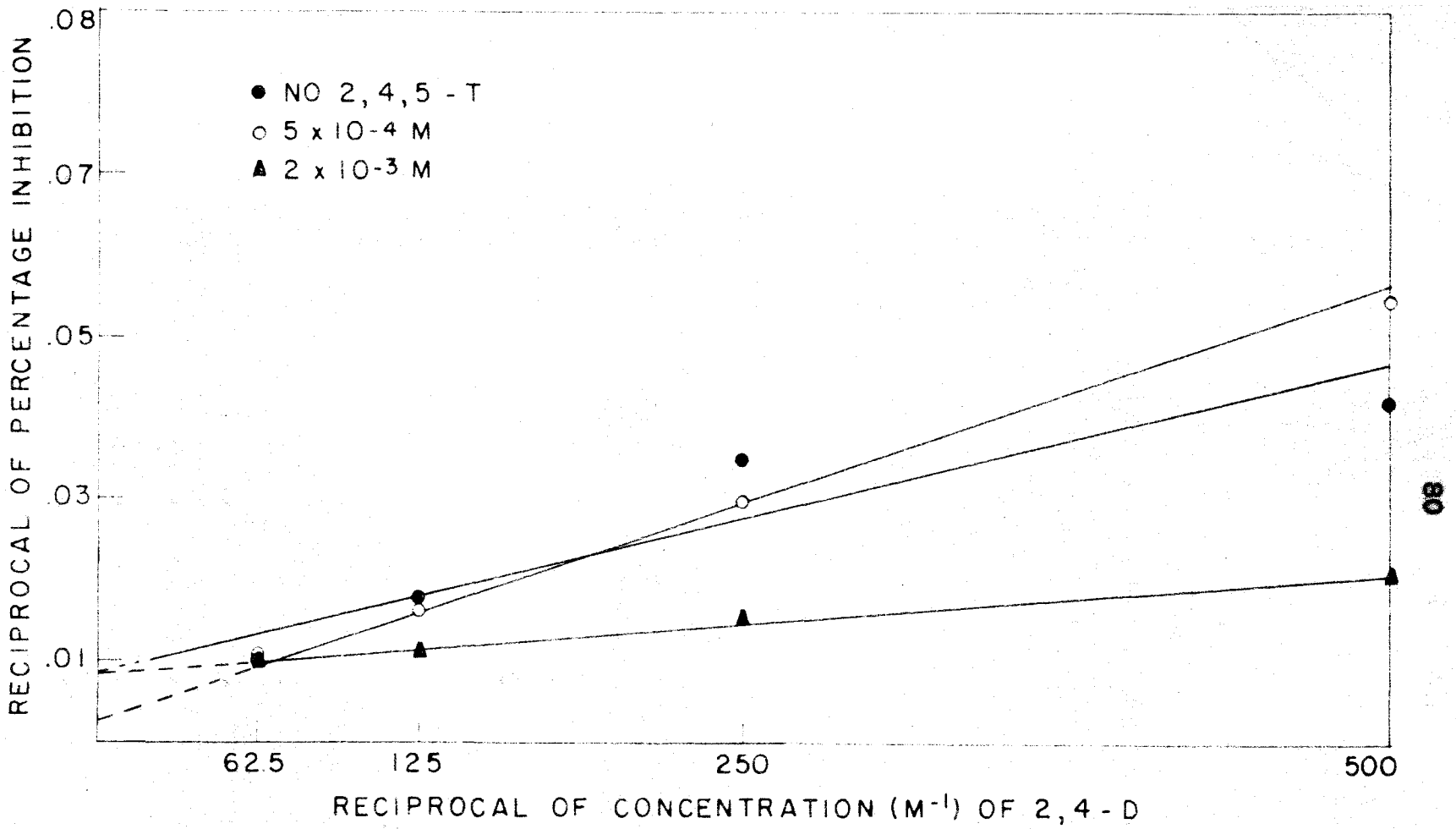

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Fig. 25. The reciprocal of inhibition of yeast growth as a function of the reciprocal of the concentration of 2,4-D at three levels of 2,4,5-T

slope of this line would undoubtedly have been greater than that of the line for the low concentration of 2,4,5-T. The slope of the line representing the high concentration was decreased significantly (probability = 95%) from the line representing the zero level, indicating a mutual action of the two compounds at this concentration. These results are contrary to those observed with soybeans, where it was found that 2,4,5-T competitively inhibited the action of 2,4-D. The mutual action observed in this experiment (Table 10) is probably an additive effect.

Similar results were noted in an experiment where mixtures of 2,4-D and coumarin were used (Table 11 and Fig. 26). The percentage inhibition data again show nothing more than additive effects between the two chemicals at the

Table 11. Percentage inhibition of yeast growth as a function of concentrations of 2,4-D and coumarin added to culture media singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of coumarin		
	None	5×10^{-4}	2×10^{-3}
None		8.5	49.7
1.5×10^{-3}	7.6	14.9	49.7
3.0×10^{-3}	12.5	18.9	49.7
6.0×10^{-3}	29.3	28.7	62.2
1.2×10^{-2}	64.6	68.6	87.5

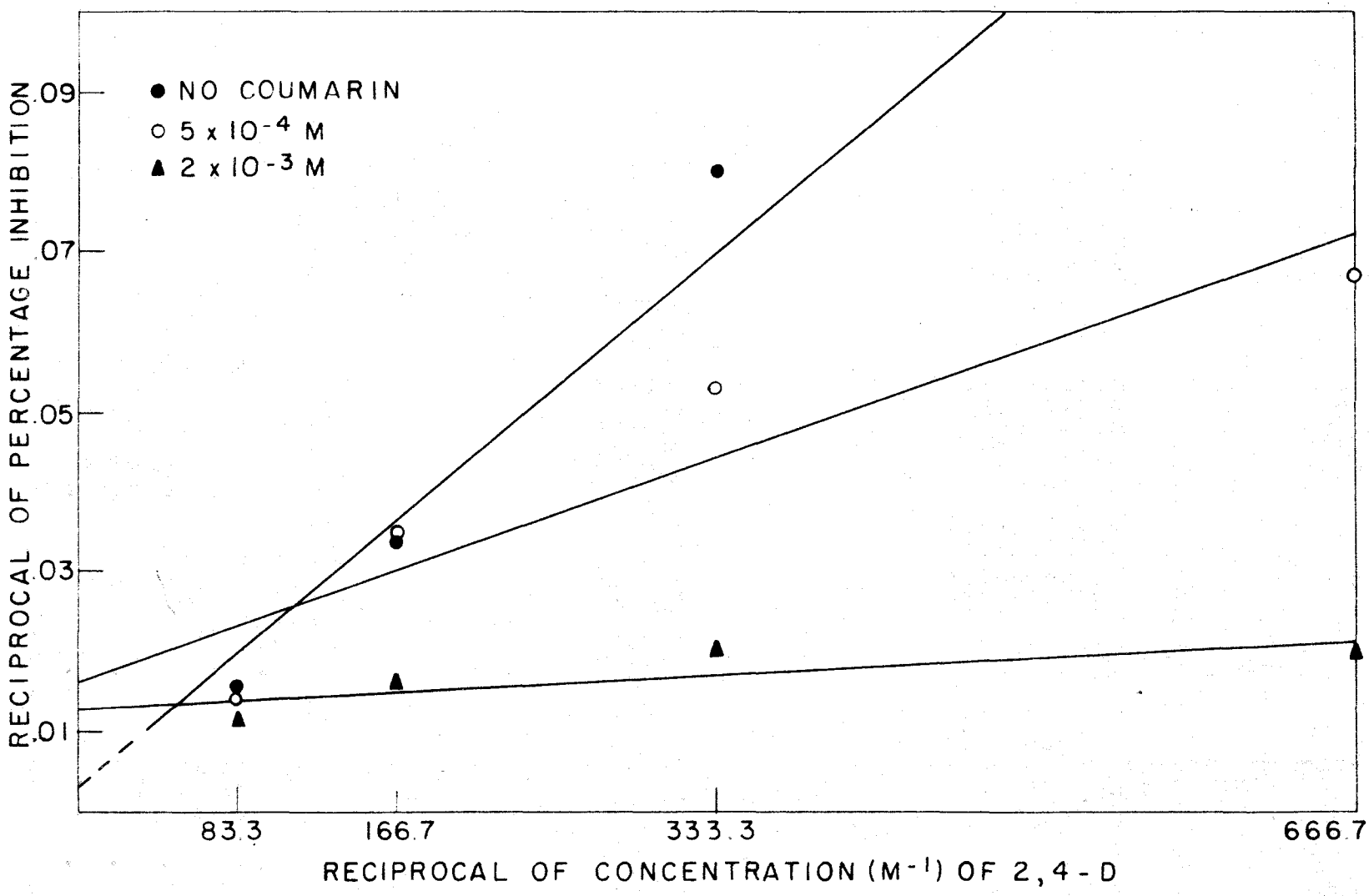

Fig. 26. The reciprocal of inhibition of yeast growth as a function of the reciprocal of the concentration of 2,4-D at three levels of coumarin

concentrations employed. The slopes of the three lines in the graph are significantly different at probability levels in excess of 95 per cent. The decreasing slopes again indicate a combined action of these two compounds.

Indoleacetic acid was found to inhibit the action of 2,4-D in soybean experiments. In yeast, however, no such competition was observed (Table 12 and Fig. 27). Rather, as in the two previous experiments, the response seemed to be a mutual action in producing inhibition, although the increased inhibition again was probably due only to additive effects. In the graph (Fig. 27) the slopes of the lines decreasing from the zero level of IAA, differ significantly from this line at probability levels in excess of 95 per cent.

Table 12. Percentage inhibition of yeast growth as a function of concentrations of 2,4-D and IAA added to culture media singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of IAA		
	None	5×10^{-4}	2×10^{-3}
None		6.1	20.7
2×10^{-3}	10.2	19.0	26.2
4×10^{-3}	22.6	26.7	34.4
8×10^{-3}	46.6	46.6	49.9
1.6×10^{-2}	87.6	84.8	86.8

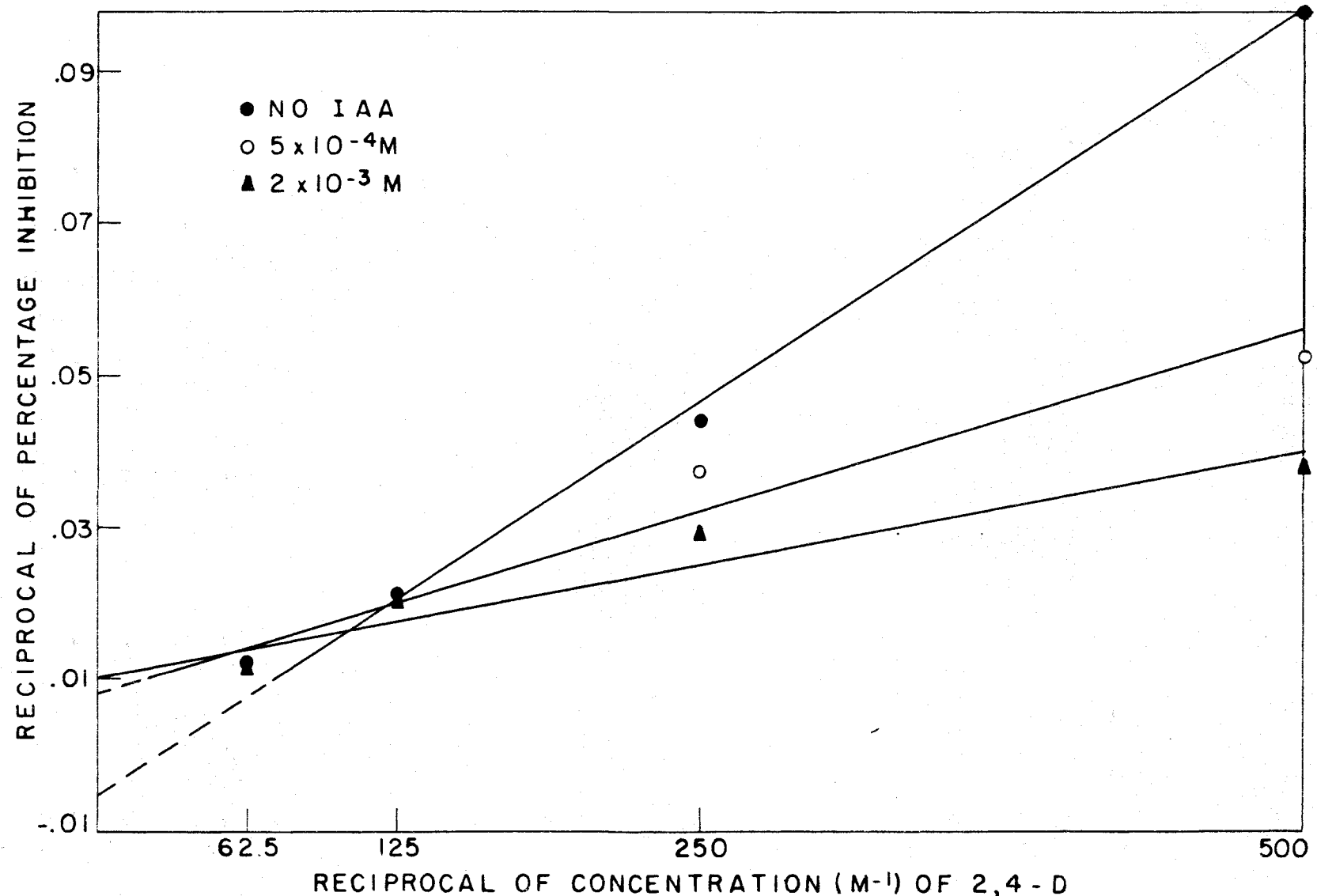

Fig. 27. The reciprocal of inhibition of yeast growth as a function of the reciprocal of the concentration of 2,4-D at three levels of IAA

Mixtures of TIBA and 2,4-D also increased inhibition of yeast growth over that observed with 2,4-D alone (Table 13 and Fig. 28). This action was similar to that observed in soybeans. The double reciprocal plot of the data shows that the experimental points deviated from the straight line to a much lesser degree than did the points in the three preceding experiments. The slope of the line representing the high concentration of TIBA differed significantly (probability = 95%) from the line representing the zero level. At the low concentration of TIBA, however, the probability level for the difference in slopes was only 80 per cent. This was probably due to the fact that TIBA alone, at this concentration (3.1×10^{-4} M), inhibited growth by only 2.3 per cent

Table 13. Percentage inhibition of yeast growth as a function of concentrations of 2,4-D and TIBA added to culture media singly and in combination

Concentration (M) of 2,4-D	Concentration (M) of TIBA		
	None	3.1×10^{-4}	1.3×10^{-3}
None	—	2.3	15.4
2×10^{-3}	12.7	16.7	49.3
4×10^{-3}	24.4	29.0	62.2
8×10^{-3}	44.8	46.2	85.1
1.6×10^{-2}	82.4	85.4	99.6

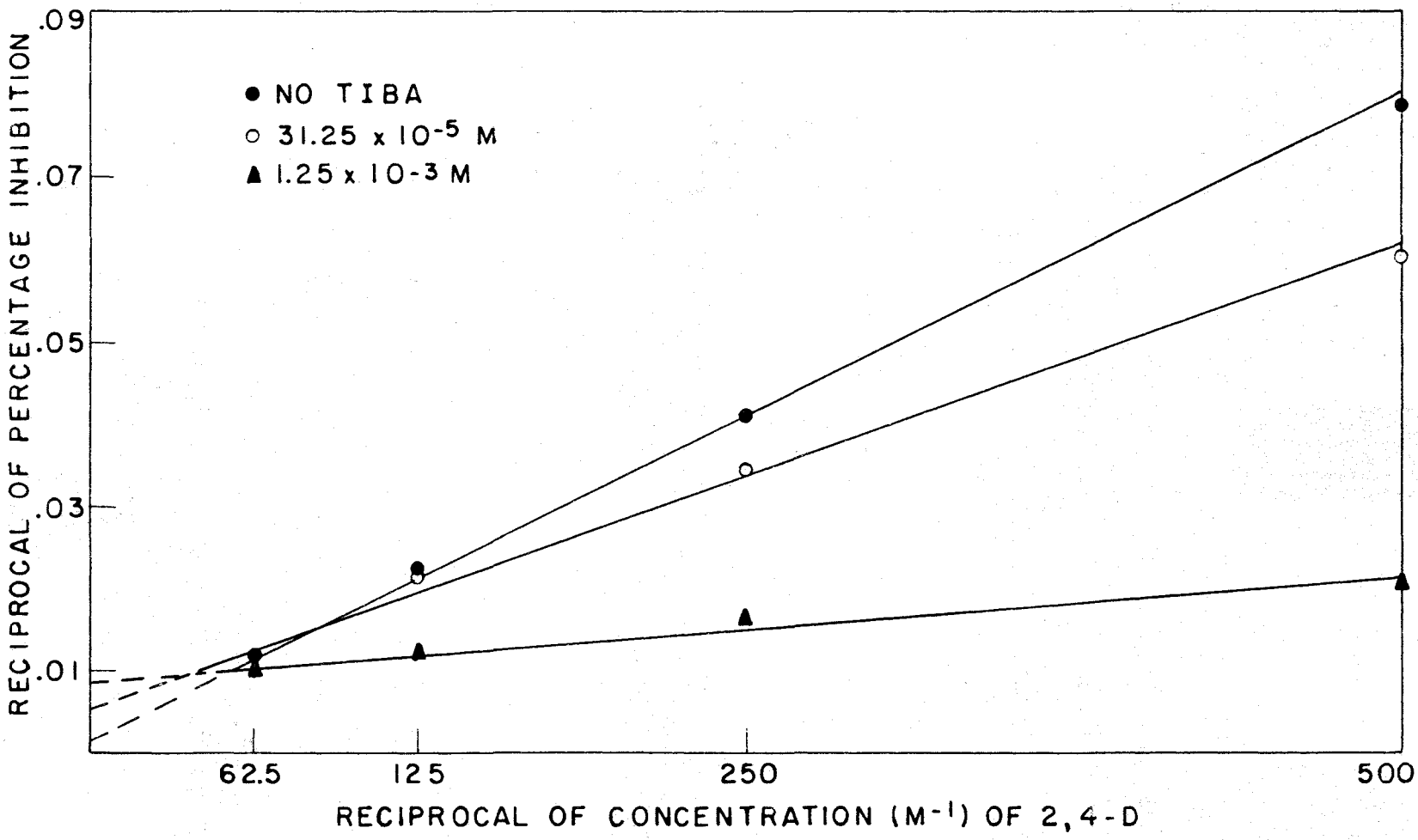

Fig. 28. The reciprocal of inhibition of yeast growth as a function of the reciprocal of the concentration of 2,4-D at three levels of TIBA

(Table 13). It will be noted that the increase in growth inhibition at this concentration is by an approximate factor of 3 per cent over that shown by 2,4-D alone, an effect which again may be considered additive. At the high concentration of TIBA (1.3×10^{-3} M), however, inhibition of growth was increased by a factor of 35 to 40 per cent (with the exception of the high concentration of 2,4-D). TIBA alone at this concentration inhibited growth only 15.4 per cent. This apparent synergistic action of TIBA has been noted in other studies on auxin interactions (3).

The results reported in this section seem to point uniformly to increased growth inhibition by the addition of the secondary compound. No decisive evidence of competitive action was obtained in these studies with yeast. It seems well to re-emphasize, however, the difference in the concentrations producing growth inhibition in yeast and soybeans, as well as the difference in the latitude of the inhibitory range. The concentration of 2,4-D, for example, producing complete inhibition of growth in yeast was nearly 15 times as great as the comparable level required in soybeans. The difference between high and low concentrations in yeast was by a factor of approximately 15 times, while the high concentration in the soybean experiment was 250 times greater than the low concentration. The data suggest a major difference in the reactions of these two organisms.

DISCUSSION

It has been postulated that plant growth inhibition proceeds from molecular reactions in which the herbicide (H) combines reversibly with some mechanism or site (M) within the cell. It is believed that this complex (HM) is then transformed into products ultimately giving rise to growth inhibition. These molecular reactions are summarized as follows:

$$H + M \rightleftharpoons HM \longrightarrow \text{Inhibition of Growth}$$

It has been shown that these relationships can be expressed quantitatively by the development of velocity reactions similar to those proposed in classical enzyme kinetics. Although this type of analysis was originally applied to auxin-induced growth, it seems logical that it should apply also to growth inhibition, especially since it is generally known that auxins are capable of producing growth inhibition at high concentrations.

The adoption of a kinetic analysis has not been the first attempt to explain growth processes on a molecular basis. Thus, it has been postulated (76) that auxin might act as a coenzyme and that some substrate might be connected through it to an enzyme controlling growth. Auxin has also been thought of as a bonding agent at an interphase between

lipoidal and aqueous materials (89). According to the ortho concept (62), auxin combines with a substrate through a position on the ring adjacent to the side-chain. This theory is basic to the idea that auxin combines at two points to the substrate, one point of attachment being the ortho position and the other point the carboxyl at the end of the side chain (26). This is the type of attachment postulated as occurring in the formation of an auxin-substrate complex, leading finally to growth (56, 57, 58).

It has been shown that the results obtained in soybean experiments with single chemicals could be satisfactorily interpreted in the light of the kinetic analysis employed. The successful application of these principles to growth inhibition responses does not provide conclusive proof of the mechanism with which the growth substance or herbicide combines. It would seem, however, that the synthetic auxins (2,4,5-T, 2,4-D and IAA) probably combine with the mechanism or site necessary to auxin-induced growth until all these available sites are completely saturated. It seems likely, then, that inhibition does not occur until these sites are completely filled and the growth substance then "spills over" onto secondary sites leading to inhibition, or combines with compounds necessary to the normal functioning of growth. Although it appears to be logical that these substances combine with the growth sites before inhibition occurs, it is less likely that such combinations occur when the growth-

inhibiting agent is not auxin-like in character (TCA, MH, AT, DCPA). These compounds do not possess the structural requirements which have been shown to be necessary for auxin action. It is probable, therefore, that these agents do not function by interfering with two-point attachment, and possible that they do not combine or react with the growth sites at all. Rather, a direct combination with secondary sites, or interference with substances required for growth is visualized. Native auxin would still react normally with the stimulatory site. Growth inhibition induced by the applied chemical, however, could mask or outweigh growth stimulation since the inhibitor is present in relatively large quantities.

The transformation of growth responses into a linear function of concentration may be of particular value in comparing, and even in screening, unknown or experimental materials which might have potential uses in applied fields. These results are somewhat similar to Blackman's findings (13) that the sigmoidal relationship between percentage mortality of a plant population and the logarithm of concentration becomes linear when percentage mortality is transformed to "probits" (25). It is believed that the kinetic analysis used here incorporates the rather obvious advantages of these linear relationships into the final results, and, at the same time, provides a good physiological interpretation

of the results.

The results obtained with single chemicals in yeast cultures have been examined in some detail. It was observed that the experimental points gave a reasonably good fit to a straight line in the double reciprocal plots and that close agreement was obtained between calculated and experimental percentage inhibition for three of the four compounds tested. The degree of confidence that can be placed in the calculated constants is less obvious, however. It is apparent, from the concentrations used, that yeast is more tolerant to these growth substances than soybeans. It can also be seen that, even though the range of concentrations covering the inhibition range is narrow, the maximum velocity of inhibition is approached rather slowly. After reaching a given level of concentration, the growth of the yeast is inhibited abruptly. This is in contrast to results obtained with soybeans, where the first portion of the response curve was considerably steeper and the maximum inhibition was approached more gradually over a wider range of concentrations. These facts are shown in the double reciprocal plots by the relatively steep slopes and the slight tendency of the points to curve as the maximum velocity is approached (cf. Figs. 12, 14 and 18). The best straight line fit to these experimental points, then, will be a compromise between the slight curvature noted and the normal variation encountered. The line

will of necessity intersect the ordinate at some point below the reciprocal of maximum velocity (0.01), and the calculation of linear regression yields a value for this point which is of no significance as an estimate of the maximum velocity. Similarly, the calculated K_m value will be an estimate of the concentration needed to attain one-half of this improbable maximum velocity rather than the true maximum velocity of 100 per cent inhibition.

These foregoing statements are not to be taken as sufficient evidence to reject the hypothesis that a kinetic analysis yields the best explanation of the results obtained. It is believed, rather, that there is insufficient evidence available to reject or accept the hypothesis for yeast. The fact remains that the double reciprocal plots and the agreement between calculated and experimental percentage inhibition may be taken as evidence supporting the hypothesis for three of the compounds reported (2,4-D, coumarin and IAA). No satisfactory explanation can be given for the divergent results noted in the 2,4,5-T experiment. It is entirely possible that the inclusion of more concentrations between the levels reported (for all the compounds) and particularly those levels at which maximum inhibition is approached, would result in a more accurate characterization of that portion of the response curve, and might also aid in attaining a calculated maximum velocity of physiological importance.

One other factor to consider before leaving this series of experiments is that of the magnitude of concentrations needed to give inhibition of yeast growth. Although it has been stated that yeast seems to be relatively tolerant to these growth substances, it may be that this tolerance is of a more far-reaching nature. It would seem that, in the yeast organism, the growth substance is not very efficient in arriving at and combining with a particular site to bring about growth inhibition. It may be possible that this inefficiency exists because part of the growth substance is being tied up or even broken down before combining with the receptor. These decomposition products may possibly be metabolized by the organism up to certain levels of saturation, after which intact molecules of the growth substance are free to react with the receptor entity. Such an interpretation might help explain the rather slow rise of the response curve and the abrupt attainment of nearly complete inhibition at a given level of the compound. It may also explain why the yeast organism, even though it is continually bathed in a solution containing the growth substance for the entire growth period, requires much higher concentrations for inhibition than do soybeans.

It was noted where mixtures of two compounds were used in the soybean experiments, that 2,4,5-T and IAA competitively inhibited the action of 2,4-D at low concentrations

of the latter. The percentage inhibition obtained with 2,4,5-T alone was considerably greater than that obtained with IAA alone, approximately 35 to 44 per cent for the former and 6 to 13 per cent for the latter. It is probable that all three of these compounds show specificity for the same site or mechanism leading to growth inhibition. It is also likely that these sites are closely related to the receptor sites causing growth stimulation, especially since IAA was able to inhibit 2,4-D action in spite of the fact that it was relatively inactive when applied alone. The results obtained with mixtures of 2,4-D and IAA are in essential agreement with the findings of Hitchcock and Zimmerman (41, 43) and Blackman and Robertson-Cuninghame (14). The former investigators concluded that IAA inhibited the action of 2,4-D. Blackman and Robertson-Cuninghame felt that the action was one of mutual interference, but that there was more than one mechanism involved. The application of the kinetic analysis to the results reported here, however, suggests that only one mechanism is involved.

The increasing inhibition noted in the remaining three experiments reported in this section is a type of response that has not been reported previously in investigations in which kinetic analysis has been employed. This increase of action of the primary compound by the addition of the secondary compound seems to indicate that the interaction of

the two is not competitive, or at most only weakly competitive. It seems logical to assume that separate mechanisms are involved where such compounds as maleic hydrazide and TIBA are used in combinations with auxins, especially since these compounds have not been observed to function as auxins in low concentrations (51, 91). Although maleic hydrazide and TIBA appear to be acting with 2,4-D and IAA to increase growth inhibition, it should be pointed out that these effects were probably only additive. A small part of the secondary compound may actually compete with the primary compound for some specific site, but such competitive action could be largely masked by the stronger inhibitive effects of the secondary compound combining with a separate site or mechanism.

The responses obtained with mixtures of compounds in yeast cultures were nearly uniform in that the initial inhibition obtained with 2,4-D alone was increased by the addition of the secondary compounds. The apparent contradiction of results obtained in soybean experiments with mixtures of 2,4,5-T and IAA with 2,4-D is probably inherent in the observed tolerance of yeast to the growth substances added. This, plus the fact that considerably more variation occurred in the yeast experiments than was noted with soybeans, seems to prevent the drawing of concrete conclusions from the data obtained. The exact nature of yeast tolerance

needs to be determined before conclusions can be formulated.

One significant fact brought out in this series of experiments was the apparent synergistic action of 2,4-D and the high concentration of TIBA. Åberg (3) has reported a similar type of response with TIBA in increasing the inhibition of the growth of flax roots induced by low concentrations of IAA. The range of concentrations over which this action was noted was from 3×10^{-9} to 10^{-8} M for IAA and 10^{-7} to 3×10^{-7} M for TIBA. Responses to similar ranges of concentration of TIBA with 2,4-D were found to be only additive. It was postulated that, since TIBA alone caused inhibition, such effects might be due to a synergistic action between TIBA and native auxin. This was offered as a possible explanation of the difference between IAA and 2,4-D, since only very low concentrations of IAA were needed to show the synergistic response.

The work thus cited forms a basis for a possible explanation of the observed responses of yeast to TIBA and 2,4-D, assuming that IAA is the native auxin of yeast. Thimann and Bonner (85) and Galston (32) have postulated that TIBA inhibits growth by excluding auxin from growth sites. Such an exclusion might be a result of TIBA combining with the site, a possibility which would agree with the findings of Waard and Florschütz (91) that TIBA in low concentrations does not promote growth. If these indeed are the true

relationships, then the action of TIBA would be to more nearly saturate the primary stimulatory site, enabling more of the 2,4-D and native auxin to react with the secondary sites, producing inhibition. It is assumed that the small amount of inhibition produced by TIBA alone is of no consequence in interfering with the inhibitory effects of 2,4-D, or that it may result from the reaction of TIBA with a site different from that of 2,4-D.

There are certain apparent limitations in the techniques employed in this investigation. Foremost and most obvious of these is the variability encountered, particularly in the greenhouse studies with soybeans. Coefficients of variability have already been noted for individual experiments. The magnitude of these coefficients is traceable in considerable part to the variable conditions under which the plants were grown. The yeast experiments were undertaken to avoid some of the more obvious variability inherent in greenhouse techniques. Although all conditions were kept as nearly constant as possible in these experiments, differences were still noted in the growth obtained in the controls of individual experiments.

Probably one of the most significant points to be noted here is the fact that the kinetic analysis could be successfully applied to growth measurements obtained in these studies in spite of these drawbacks. It can be concluded

that this analysis may well serve to characterize growth inhibition induced in plants by certain herbicides and growth substances.

SUMMARY

Several herbicides and growth substances were applied singly and in combinations at varying concentrations to soybean seedlings and yeast cultures in an attempt to characterize accurately the growth inhibition responses obtained. The growth measurements were subjected to a kinetic analysis in analogy with that proposed for enzyme systems and later modified to include auxin-induced growth.

Growth inhibition of soybean seedlings induced by single applications of 2,4-D, DCPA, 2,4,5-T, TCA, AT, IAA, and MH, satisfactorily supported the hypothesis inherent in the kinetic analysis. It is suggested that calculated K_m values may be of value in determining relative affinities of these compounds for sites or mechanisms within the plant leading to growth inhibition.

Results obtained by single additions of 2,4-D, coumarin, and IAA to yeast cultures showed satisfactory agreement to straight line reciprocal plots and between calculated and experimental percentage inhibition. The calculated constants were of little value, however, in characterizing or comparing compounds, since the calculated maximum velocities obtained for 2,4-D, coumarin and 2,4,5-T were in excess of the possible 100 per cent. Results obtained with 2,4,5-T were not satisfactorily interpreted by this analysis.

Growth inhibition of soybean seedlings induced by low concentrations of 2,4-D was competitively inhibited by 2,4,5-T and IAA. The competitive action was not evident at higher concentrations of 2,4-D. The effect of TIBA and MH was to increase, apparently additively, inhibition caused by 2,4-D and IAA. It is suggested that any competitive effects that might have existed were largely masked by the stronger inhibitive effects of the secondary compound (TIBA and MH) on an inhibitory site separate from that of the primary compound (2,4-D or IAA).

Growth inhibition of yeast cultures induced by 2,4-D was found to be increased additively by 2,4,5-T, coumarin, and IAA, and synergistically by TIBA. The apparently contradictory results obtained with 2,4,5-T and IAA in combination with 2,4-D between yeast and soybean experiments are thought to be a function of possible differential utilization of growth substances by yeast.

LITERATURE CITED

1. Åberg, B. On auxin antagonists and synergists in root growth. Physiol. Plantarum 3: 447-461. 1950.

2. _____. On the effects of weak auxins and antiauxins upon plant growth. Physiol. Plantarum 5: 305-317. 1952.

3. _____. On the interaction of 2,3,5-triiodobenzoic acid and maleic hydrazide with auxins. Physiol. Plantarum 6: 277-291. 1953.

4. _____. The interaction of some auxin antagonists and 2,4-D in root growth. Physiol. Plantarum 4: 627-640. 1951.

5. Allard, R. W., De Rose, H. R. and Swanson, C. P. Some effects of plant growth-regulators on seed germination and seedling development. Bot. Gaz. 107: 575-583. 1946.

6. Audus, L. J. Auxin antagonists and synergists. A critical approach. New Phytol. 53: 461-469. 1954.

7. _____. Studies on the phytostatic action of 2,4-dichlorophenoxyacetic acid and coumarin. The reversibility of root-growth inhibitions. New Phytol. 47: 196-219. 1948.

8. _____. The mechanism of auxin action. Biol. Rev. Cambridge Philos. Soc. 24: 51-93. 1949.

9. _____, and Quastel, J. H. Coumarin as a selective phytocidal agent. Nature (London) 159: 320-324. 1947.

10. _____ and Shipton, M. E. 2,4-dichloroanisole-auxin interactions in root-growth. Physiol. Plantarum 5: 430-455. 1952.

11. Bennet-Clark, T. A. and Kefford, N. P. The extension growth-time relationship for Avena coleoptile sections. Jour. Exp. Bot. 5: 293-304. 1954.

12. Berger, J. and Avery, G. S., Jr. The mechanism of auxin action. Science 98: 454-455. 1943.

13. Blackman, G. E. Studies on the principles of phytotoxicity. I. The assessment of relative toxicity. Jour. Exp. Bot. 3: 1-28. 1952.

14. _____ and Robertson-Cuninghame, R. C. Interactions in the physiological effects of growth substances on plant development. Jour. Exp. Bot. 5: 184-203. 1954.

15. _____, Templeman, W. G. and Halliday, D. J. Herbicides and selective phytotoxicity. Ann. Rev. Plant Physiol. 2: 199-230. 1951.

16. Brian, R. C. and Rideal, E. K. On the action of plant growth regulators. Biochim. et Biophys. Acta 9: 1-18. 1952.

17. Burström, H. Studies on growth and metabolism of roots. XI. The influence of auxin and coumarin derivatives on the cell wall. Physiol. Plantarum 7: 548-559. 1954.

18. Crafts, A. S. A theory of herbicidal action. Science 108: 85-86. 1948.

19. _____. Herbicides. Ann. Rev. Plant Physiol. 4: 253-282. 1953.

20. _____. Weed control in the tropics. Science 107: 196-197. 1948.

21. Currier, H. B. Responses of plant cells to herbicides. Plant Physiol. 24: 601-609. 1949.

22. Dancaster, E. A. Catalysts for sodium chlorate in weed destruction. Nature (London) 150: 737-738. 1942.

23. Ennis, W. B., Jr. and Boyd, F. T. The response of kidneybean and soybean plants to aqueous spray applications of 2,4-dichlorophenoxyacetic acid with and without Carbowax. Bot. Gaz. 107: 552-559. 1946.

24. Eyster, H. C. Effect of auxins on the action of diastase in vitro. Plant Physiol. 21: 68-74. 1946.

25. Finney, D. J. Probit Analysis. Cambridge at the University Press. 1952.

26. Foster, R. J., McRae, D. H. and Bonner, J. Auxin-induced growth inhibition a natural consequence of two-point attachment. Proc. Natl. Acad. Sci. 38: 1014-1022. 1952.

27. Freed, V. H. Herbicide mechanism. Mode of action other than aryl oxyalkyl acids. Jour. Agric. Food Chem. 1: 47-51. 1953.

28. Freiberg, S. R. Effects of an exogenous growth regulator on proteolytic enzymes of the soybean plant. Science 115: 674-675. 1952.

29. French, R. C. and Beevers, H. Respiratory and growth responses induced by growth regulators and allied compounds. Amer. Jour. Bot. 40: 660-666. 1953.

30. Galston, A. W. Indoleacetic-nicotinic acid interactions in the etiolated pea plant. Plant Physiol. 24: 577-586. 1949.

31. _____. Synergism between indoleacetic and nicotinic acids in a plant growth inhibition. Jour. Biol. Chem. 169: 465-466. 1947.

32. _____. The effect of 2,3,5-triiodobenzoic acid on the growth and flowering of soybeans. Amer. Jour. Bot. 34: 356-360. 1947.

33. Goldacre, P. L. On the mechanism of action of 2,4-dichlorophenoxyacetic acid. Australian Jour. Sci. Res., Sect. B, 2: 154-156. 1949.

34. _____, Galston, A. W. and Weintraub, R. L. The effect of substituted phenols on the activity of the indoleacetic acid oxidase of peas. Arch. Biochem. Biophys. 43: 358-373. 1953.

35. Grace, N. H. Physiological curve of response to phytohormones by seeds, growing plants, cuttings, and lower plant forms. Can. Jour. Res., Sect. C, 15: 538-546. 1937.

36. Hance, F. E. Recent developments in weed control. Science 108: 278-279. 1948.

37. _____. The factor of synergism in chemical weed control. Hawaiian Planter's Record 44: 263-272. 1940.

38. Hansch, C. and Muir, R. M. The ortho effect in plant growth regulators. Plant Physiol. 25: 389-393. 1950.

39. Hartman, R. T. and Price, W. C. Synergistic effect of plant growth substances and Southern Bean Mosaic virus. Amer. Jour. Bot. 37: 820-828. 1950.

40. Hauser, E. W. Absorption of 2,4-dichlorophenoxyacetic acid by soybean and corn plants. J. Amer. Soc. Agron. 47: 32-36. 1955.

41. Hitchcock, A. E. Additive and inhibitive effects resulting from treatment of tomato seedlings with indoleacetic acid in combination with 2,4-dichlorophenoxyacetic acid. (Abstract) Torrey Bot. Club, Sect. B, 79: 260-261. 1952.

42. _____ and Zimmerman, P. W. Activation of 2,4-D by various adjuvants. Contrib. Boyce Thompson Inst. 15: 173-193. 1948.

43. _____ and _____. Responses of tomato plants to treatments with 2,4-dichlorophenoxyacetic acid in combination with indoleacetic and certain other compounds. Contrib. Boyce Thompson Inst. 17: 35-55. 1952.

44. Hoffman, O. L. Inhibition of auxin effects by 2,4,6-trichlorophenoxyacetic acid. Plant Physiol. 28: 622-628. 1953.

45. Housley, S., Bentley, J. A. and Bickle, A. S. Studies on plant growth hormones. III. Application of enzyme reaction kinetics to cell elongation in the Avena coleoptile. Jour. Exp. Bot. 5: 373-388. 1954.

46. Ingestad, T. Kinetic aspects on the growth-regulating effects of some phenoxy acids. Physiol. Plantarum 6: 797-803. 1953.

47. King, G. S. 2,4-D herbicides for water hyacinths. (Abstract) Amer. Jour. Bot. 33: 837. 1946.

48. Koepfli, J. B., Thimann, K. V. and Went, F. W. Phytohormones: Structure and physiological activity. I. Jour. Biol. Chem. 122: 763-780. 1938.

49. Kvamme, O. J., Clagett, C. D. and Treumann, W. B. Kinetics of the action of the sodium salt of 2,4-dichlorophenoxyacetic acid on the germ lipase of wheat. Arch. Biochem. 24: 321-328. 1949.

50. Leopold, A. C. Auxins and Plant Growth. Univ. of Calif. Press. Berkeley. 1955.

51. _____ and Klein, W. H. Maleic hydrazide as an antiauxin. Physiol. Plantarum 5: 91-99. 1952.

52. Lineweaver, H. and Burk, D. The determination of enzyme dissociation constants. Jour. Amer. Chem. Soc. 56: 658-666. 1934.

53. Linser, H. and Kaindl, K. The mode of action of growth substances and growth inhibitors. Science 114: 69-70. 1951.

54. Lucas, E. H. and Hamner, C. L. Modification of the physiological action of the sodium salt of 2,4-dichlorophenoxyacetic acid by simultaneous application of plant extracts and by pH changes. Mich. Agric. Expt. Sta. Quar. Bull. 29: 256-262. 1947.

55. Mangual, J. C. Increase of herbicidal action of concentrate 40 and oil emulsion by 2,4-D. Science 107: 66. 1948.

56. McRae, D. H. and Bonner, J. Chemical structure and antiauxin activity. Physiol. Plantarum 6: 485-510. 1953.

57. _____ and _____. Diortho substituted phenoxyacetic acids as antiauxins. Plant Physiol. 27: 834-838. 1952.

58. _____, Foster, R. J. and Bonner, J. Kinetics of auxin interaction. Plant Physiol. 28: 343-355. 1953.

59. Michaelis, L. and Menten, M. L. Die Kinetik der Invertwirkung. Biochem. Zeitschr. 49: 333-369. 1913.

60. Muir, R. M. and Hansch, C. On the mechanism of action of growth regulators. Plant Physiol. 28: 218-232. 1953.

61. _____ and _____. The relationship of structure and plant-growth activity of substituted benzoic and phenoxyacetic acids. Plant Physiol. 26: 369-374. 1951.

62. _____, _____ and Gallup, A. H. Growth regulation by organic compounds. Plant Physiol. 24: 359-366. 1949.

63. Naylor, A. W. and Davis, E. A. Maleic hydrazide as a plant growth inhibitor. Bot. Gaz. 112: 112-126. 1950.

64. Nolla, J. A. B. The control of grass weeds in sugar cane fields in Puerto Rico. Science 108: 112-113. 1948.

65. Norman, A. G., Minarik, C. E. and Weintraub, R. L. Herbicides. Ann. Rev. Plant Physiol. 1: 141-168. 1950.

66. Northen, H. T. Relation of dissociation of cellular proteins by auxins to growth. Bot. Gaz. 103: 668-683. 1942.

67. Osborne, D. J. A synergistic interaction between 3-indolylacetonitrile and 3-indolylacetic acid. Nature (London) 170: 210. 1952.

68. Paleg, L. G. and Muir, R. M. Surface activity as related to physiological activity of plant-growth regulators. Plant Physiol. 27: 285-292. 1952.

69. Parry, D. W. A growth interaction between beta-indolylacetic acid and thiourea (thiocarbamide). Nature (London) 170: 1074. 1952.

70. Pool, E. L. Effect of phytohormones on the growth of certain microorganisms. Unpublished M. S. Thesis. Ames, Iowa, Iowa State College Library. 1949.

71. Rhodes, A. and Ashworth, R. de B. Mode of action of growth regulators in plants. Nature (London) 169: 76-77. 1952.

72. Robbins, W. W., Crafts, A. S. and Raynor, R. N. Weed Control. McGraw-Hill Book Co. Inc. New York. 1942.

73. Schoene, D. L. and Hoffman, O. L. Maleic hydrazide, a unique growth regulant. Science 109: 588-590. 1949.

74. Sen, G. and Woodford, E. K. Effects of TCA acid on the extension growth of root and shoot segments of Pisum sativum. Nature (London) 171: 936-937. 1953.

75. Shantz, E. M., Steward, F. C., Smith, M. S. and Wain, R. L. Investigations on the growth and metabolism of plant cells. VI. Growth of potato tuber tissue in culture: The synergistic action of coconut milk and some synthetic growth-regulating compounds. Ann. Botany 19: 49-58. 1955.

76. Skoog, F., Schneider, C. L. and Malan, P. Interactions of auxins in growth and inhibition. Amer. Jour. Bot. 29: 568-576. 1942.

77. Spear, I. and Thimann, K. V. The effect of onion juice on the growth response to auxin. Plant Physiol. 24: 587-600. 1949.

78. Steward, F. C. and Caplin, S. M. A tissue culture from potato tuber: The synergistic action of 2,4-D and of coconut milk. Science 113: 518-520. 1951.

79. Stewart, W. S., Riehl, L. A. and Erickson, L. C. Effects on citrus of 2,4-D used as an amendment to oil sprays. Jour. Econ. Ent. 45: 658-668. 1952.

80. Summerford, W. T. Synergism and synergists. Review of synergism among halogen-containing insecticides and halogen-containing synergists. Jour. Agric. Food Chem. 2: 310-327. 1954.

81. Swanson, C. R. Responses of yeast to 2,4-D. Unpublished Ph. D. Thesis. Ames, Iowa, Iowa State College Library. 1953.

82. Sweeney, B. M. and Thimann, K. V. The effect of auxins on protoplasmic streaming in Avena. III. Jour. Gen. Physiol. 25: 841-854. 1942.

83. Tang, Y. W. and Bonner, J. Enzymatic inactivation of indoleacetic acid. (Abstract) Amer. Jour. Bot. 33: 839. 1946.

84. Thimann, K. V. The role of ortho-substitution in the synthetic auxins. Plant Physiol. 27: 392-404. 1952.

85. _____ and Bonner, W. D. The action of tri-iodobenzoic acid on growth. Plant Physiol. 23: 158-161. 1948.

86. Van Overbeek, J. Use of synthetic hormones as weed killers in tropical agriculture. Econ. Bot. 1: 446-459. 1947.

87. _____, Blondeau, R. and Horne, V. Difference in activity between 2,4-dichlorophenoxyacetic acid and other auxins and its significance in herbicidal action. Plant Physiol. 26: 687-696. 1951.

88. Veldstra, H. Researches on plant growth substances V. Relation between chemical structure and physiological activity II. Contemplations on place and mechanism of the action of growth substances. Enzymologia 11: 137-163. 1944.

89. _____. The relation of chemical structure to biological activity in growth substances. Ann. Rev. Plant Physiol. 4: 151-198. 1953.

90. _____ and Booij, H. L. Researches on plant growth regulators XVII. Structure and activity. On the mechanism of the action III. Biochim. et Biophys. Acta 3: 278-312. 1949.

91. Waard, J. de and Florschütz, P. A. On the interaction of 2,3,5-triiodobenzoic acid and indole-3-acetic acid in growth processes. Proc. K. Nederl. Akad. Wet. 51: 1317-1321. 1948.

92. Weintraub, R. L. 2,4-D. Mechanisms of action. Jour. Agric. Food Chem. 1: 250-254. 1953.

93. Went, F. W. Differences in physiological activity of plant-growth substances. Science 108: 681-682. 1948.

94. _____. Phytohormones: Structure and physiological activity. II. Arch. Biochem. 20: 131-136. 1949.

95. West, F. R. and Henderson, J. H. M. A turbidimetric method for determining the effect of 2,4-D upon the growth of yeast. Science 107: 604. 1948.

96. Wildman, S. G. and Gordon, S. A. The release of auxin from isolated leaf proteins of spinach by enzymes. Proc. Natl. Acad. Sci. 28: 217-228. 1942.

97. Wood, J. W., Mitchell, J. W. and Irving, G. W., Jr. Translocation of a radioactive plant-growth regulator in bean and barley plants. Science 105: 337-339. 1947.

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APPENDIX

Table 14. Inhibition of growth (soybean seedlings) by 2,4-D. Growth measured as increase of fresh weight above primary leaves 11 days after treatment

Conc. M x 10^{-4}	Growth (fresh weights-g) Replicates 1	2	3	4	5	6	7	8	Mean	% inhibition
0.00	3.15	3.04	3.36	2.98	3.15	3.36	3.76	1.99	3.10	
0.32	2.11	2.97	2.62	2.21	2.16	2.22	2.50	2.65	2.43	21.6
0.64	2.15	2.16	2.24	2.26	1.74	2.20	1.86	2.03	2.08	32.9
1.28	1.22	2.03	2.13	1.79	1.16	1.61	1.78	0.63	1.54	50.3
2.56	0.99	1.12	1.16	0.82	1.13	0.80	0.72	1.00	0.97	68.7
5.12	0.34	0.20	0.27	0.60	0.21	0.52	0.45	0.47	0.38	87.7
10.24	0.09	0.08	0.20	0.20	0.14	0.09	0.21	0.16	0.15	95.2
20.48	0.16	0.06	0.09	0.00	0.06	0.05	0.06	0.00	0.06	98.1

Table 15. Inhibition of yeast growth by 2,4-D. Growth measured as increase of turbidity of cultures 15 hours after inoculation

Conc. M x 10^{-3}	Growth (optical density x500) 1	2	Mean	Percentage inhibition
0.0	181	181	181	
2.0	157	157	157	13.3
4.0	136	144	140	22.7
6.0	121	118	120	34.0
8.0	96	94	95	47.5
12.0	49	47	48	73.5
16.0	16	20	18	90.1
32.0	8	11	10	95.2

Table 16. Inhibition of growth (soybean seedlings) by single and combined concentrations of TIBA and 2,4-D. Growth measured as increase of fresh weight above primary leaves 11 days after treatment

Concentration TIBA Mx10^{-5}	Concentration 2,4-D Mx10^{-5}	Growth (fresh weights-g) Replicates 1	2	3	4	5	6	Mean	% inhibition
0.0	0.0	4.06	5.51	5.46	4.85	5.39	4.23	4.92	
0.0	3.2	4.14	3.68	3.68	3.59	3.06	2.84	3.50	28.9
0.0	6.4	2.70	2.56	2.33	2.60	2.97	1.91	2.51	49.0
0.0	12.8	1.44	1.57	0.90	1.55	0.99	0.67	1.19	75.8
0.0	25.6	0.95	0.81	1.10	0.69	0.90	1.42	0.98	80.1
0.25	0.0	5.33	4.81	4.67	3.95	3.58	3.69	4.34	11.8
0.25	3.2	4.55	2.97	2.44	5.11	3.18	2.96	3.53	28.2
0.25	6.4	1.55	2.55	3.35	4.07	2.87	2.95	2.89	41.2
0.25	12.8	1.87	1.39	1.04	1.63	2.14	1.84	1.65	66.5
0.25	25.6	1.28	0.62	1.10	0.67	0.99	0.97	0.94	80.9
1.0	0.0	5.11	4.02	4.47	3.57	3.58	3.91	4.11	16.5
1.0	3.2	4.02	1.73	3.41	2.77	2.70	2.84	2.91	40.9
1.0	6.4	2.08	1.24	1.96	1.71	2.23	2.03	1.87	62.0
1.0	12.8	1.12	0.75	0.87	1.35	1.02	1.09	1.03	79.1
1.0	25.6	0.67	0.75	0.38	0.68	0.48	1.57	0.75	84.8
4.0	0.0	3.35	2.72	2.53	3.22	3.10	2.70	2.94	40.2
4.0	3.2	2.91	2.06	2.90	2.56	2.88	1.65	2.49	49.4
4.0	6.4	1.50	2.50	2.08	2.90	1.76	1.47	2.03	58.7
4.0	12.8	0.89	0.97	0.88	1.23	0.75	0.33	0.84	82.9
4.0	25.6	0.48	0.36	0.22	0.64	0.36	0.46	0.42	91.5

Table 17. Inhibition of yeast growth by single and combined concentrations of TIBA and 2,4-D. Growth measured as increase of turbidity of cultures 15 hours after inoculation

Concentration TIBA M x 10^{-4}	Concentration 2,4-D M x 10^{-3}	Growth (optical density x500) 1	2	Mean	Percentage inhibition
0.0	0.0	220	222	221	
0.0	2.0	190	196	193	12.7
0.0	4.0	164	170	167	24.4
0.0	8.0	126	118	122	44.8
0.0	16.0	38	40	39	82.4
3.1	0.0	210	222	216	2.3
3.1	2.0	185	183	184	16.7
3.1	4.0	157	157	157	29.0
3.1	8.0	120	118	119	46.2
3.1	16.0	32	32	32	85.4
12.5	0.0	188	186	187	15.4
12.5	2.0	114	110	112	49.3
12.5	4.0	84	83	84	62.2
12.5	8.0	34	32	33	85.1
12.5	16.0	8	8	8	99.6